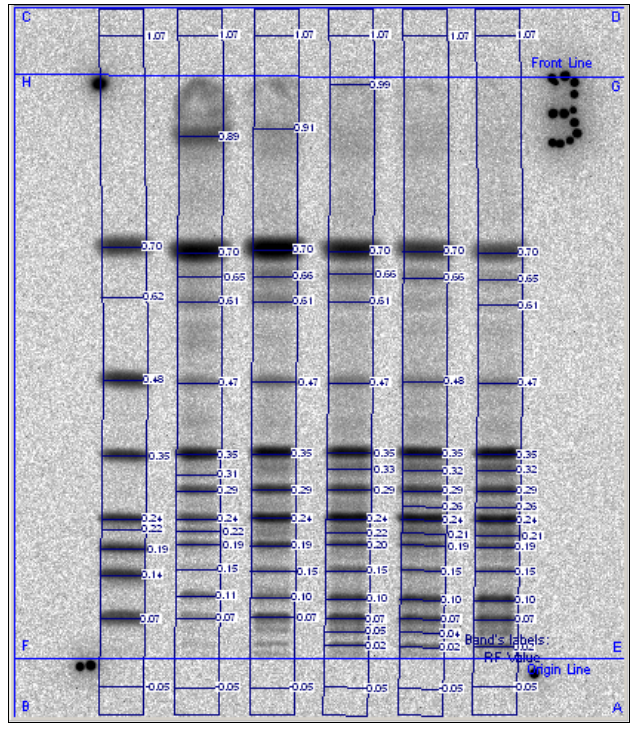
raytest

AIDA 1D Thin Layer Chromatography

User's Manual



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Introduction 1

Overview

The AIDA 1D Thin Layer Chromatography module (henceforth 1D TLC) facilitates the analysis of 1D applications using quantification and retention factor (RF) value evaluation. The first step in the evaluation is to set the TLC plate overlay and the origin and front line, followed by detecting/marking of the lanes and bands. Then the quantities are normalized by setting quantity standards, as applicable. The result is a fully evaluated image where all the information can be collected in a comprehensive printout.

To comply to GPL standards, AIDA 1D TLC provides record keeping features in addition to its 1D TLC features. All changes to a previously performed evaluation can be recorded and are saved to a AIDA Lock File (.alf). The system administrator can fully configure the behavior of the GLP supporting tools, requiring for example to prompt the user to explicitly account for all modification of existing evaluations.

Starting 1D TLC

To select the 1D TLC mode:

1 Click the **Evaluation** button in the toolbar.

A dialog box appears offering you a list of available evaluation modes.

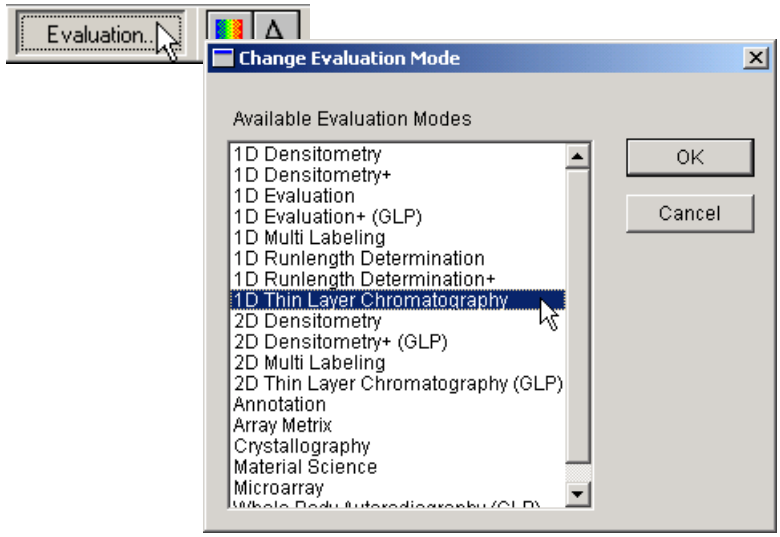


Figure 1-1 Changing Evaluation Mode

The list entries reflect the installed AIDA application modules.

2 Select the **1D Thin Layer Chromatography** list item und click **OK**.

Note – This evaluation mode is only available if you purchased the AIDA 1D TLC module. Note further that there are two possible 1D TLC evaluation modes in AIDA 1D TLC, one with GLP features (indicated by the acronym “GLP” in parentheses) and the other without those functionality. The **1D Thin Layer Chromatography (GLP)** evaluation mode is only available after establishing access control by applying the user registration (see the “Create User Registration” section below).

Create User Registration

Access control to AIDA might be required when working under Good Laboratory Practice (GLP) conditions. AIDA provides three different types of users, a System Administrator, Administrators and normal Users.

The *System Administrator* is a fixed account in AIDA which cannot be changed or deleted. When no password is set for the System Administrator, the user registration in AIDA is deactivated.

Administrators have the permission to create, modify and delete users or administrators. For GLP relevant modifications of the program settings, general administrator rights are required in AIDA.

Users can modify their own password. They have access to the normal functionality of AIDA.

When the user registration is activated, you must enter your user name and password before you get access to the program.

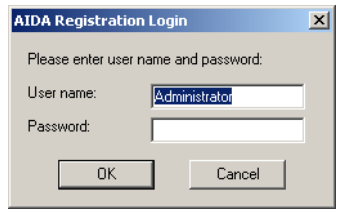


Figure 1-2 AIDA Registration Login Dialog Box

To activate the user registration in AIDA the first time, you must enter a password for the system administrator account.

To enter the password for the system administrator account:

1. Choose **Create User Registration** from the **Options** menu.

   The **AIDA Registration User Management Setup** dialog box appears.

2. Type a password for the system administrator account in the **Password** text field of the **Aida Registration User Management Setup** dialog box and confirm the new password by reentering it in the **Confirm Password** field.

AIDA Registration User Management Setup
This dialog will activate the user management of this program.
From now on, the program will require a user login on each start.
Please enter an administrator password for the user management. Be sure not to forget this password!
Password:
****
Confirm password:
****
OK
Cancel

Figure 1-3 Entering Administrator Password

3 Click **OK** to apply the changes.

Now the user registration is activated and only registered operators have access to the program.

When the user registration is activated and the operator has administrator privileges, the **User Registration** command is selectable from the **Options** menu.

On choosing this option, the **AIDA Registration User Management** dialog box appears, allowing you to add new users or to modify or delete existing user accounts.

Figure 1-4 AIDA Registration User Managament Dialog Box

To add new users:

1 On the **AIDA Registration User Management** dialog box click **Add** to open the **Add User** dialog box.

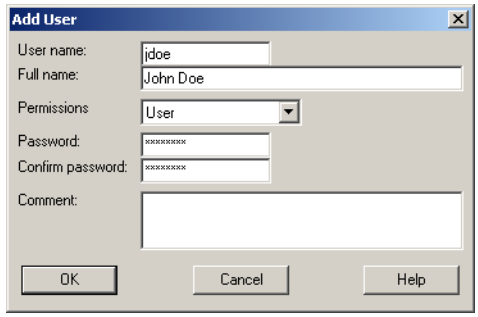


Figure 1-5 Adding Users

2 Type a user name, full name, and password for the new user, and confirm the password by reentering it in the **Confirm password** field.

A **User name** can consist of up to 31 characters and must be unique in the registration database.

The **Full name** is used whenever the program has to display the name of the operator (e.g. on print outs). It cannot be longer than 63 characters.

The user's **Password** must contain at least 4 characters (maximum 8 characters).

3 Specify the user's permission level.

Currently two permission levels, **Administrator** and **User** (see above), are available.

4 Enter an optional comment for the account.

The **Comment** for a user account appears only within the user registration database.

5 Click **OK** to apply the changes.

To modify an existing user account:

1 On the **AIDA Registration User Management** dialog box click **Modify** to open the **Modify User** dialog box.

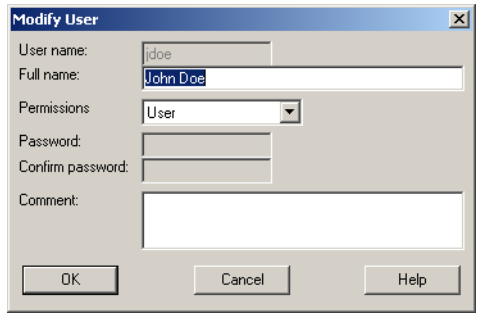


Figure 1-6 Modifying Users

2 Edit the database entries you want to change.

3 Click **OK** to confirm the changes.

If the operator has user permission, the **Change Password** option instead of the **User Registration** option is selectable from the **Options** menu.

To change your password:

1 Choose **Change Password** from the **Options** menu.

The **Change AIDA Registration Password** dialog box appears.

Figure 1-7 Changing Password

2 Type your current password in the **Old password** text box.

3 Type your new password in the **New password** text field and confirm it by reentering it in the **Confirm password** field.

4 Click **OK** to apply the changes.

At the end of the session you can choose to log on as a different user instead of exiting the program.

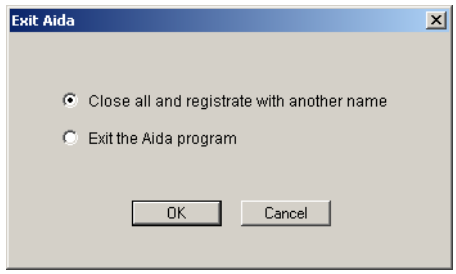


Figure 1-8 Exit Aida Dialog Box

Using 1D TLC 2

Data Windows in 1D TLC

In addition to the AIDA standard data windows **Image** and **Histogram** the following data windows are available in the **1D TLC** mode:

- Profiles
- Quantity Calibration
- Audit Trail

You can access these data windows by choosing the appropriate entry (**Profiles**, **Quantity Calibration** and **Audit Trail** respectively) from the **View** menu.

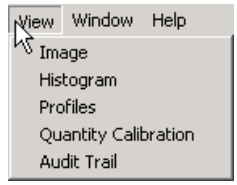


Figure 2-1 View Menu

Image Window in 1D TLC

All data are evaluated from the **Image** window. Although it might not always be on top, it is always open. The title bar of the **Image** window shows the full file path of the image.

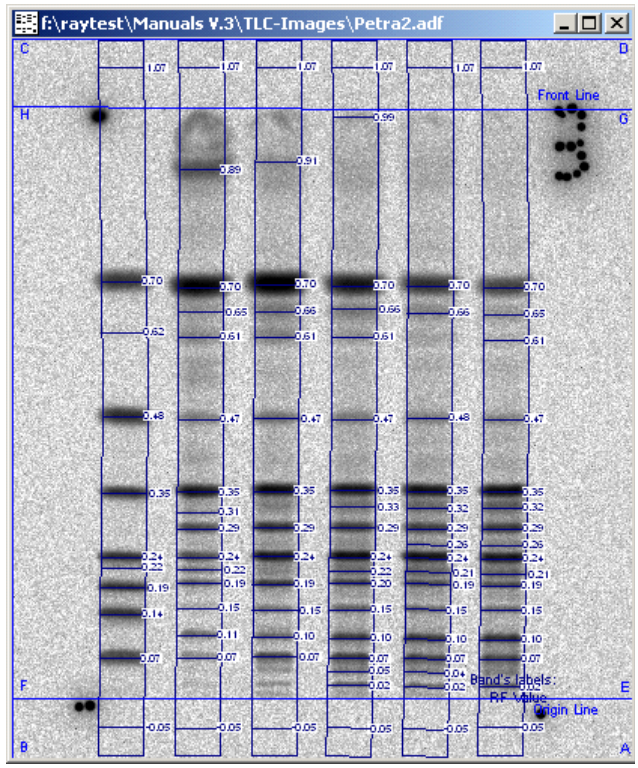


Figure 2-2 Image Window Example

You can copy the content of the **Image** window to the clipboard using the **Copy Content** command of the **Edit** menu. See the Export Image section in the AIDA Image Analyzer Basic Concepts and Features User's Manual for further explanation.

Contextual Menu of the Image Window

In 1D TLC, the contextual menu of the **Image** window looks slightly different depending on the type of overlay, you have chosen:

Lane Selection:
If you have selected one or more lanes, the contextual menu, that opens after right-clicking the respective object, provides you with the possible commands for this specific selection. These include commands for editing and labeling profiles (lanes).

Band Selection:
If you have selected a single band, the contextual menu, that opens after right-clicking the respective object, provides you with the possible commands for this type of selection. These include commands for editing and labeling profiles (lanes) as well as commands for editing peaks (bands). Note that if you select multiple bands, the peak editing command of the contextual menu is turned off.

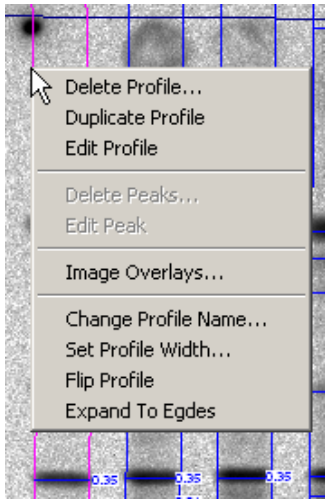


Figure 2-3 Contextual Menu (Lane Selected)

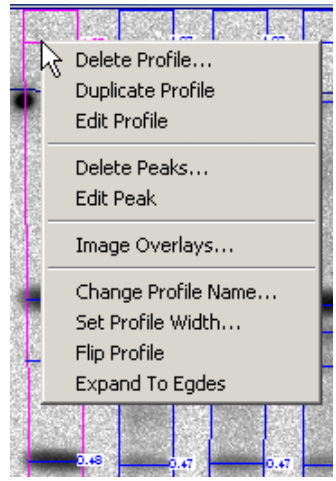


Figure 2-4 Contextual Menu (Band Selected)

The **Delete Profiles** and **Delete Peaks** commands are only executed for the selected lane(s) and selected peaks respectively.

With the **Duplicate Profile** commands you can insert another profile, identical to the currently selected one.

Edit Profile allows you to reshape the currently selected profile.

The currently selected peak area (band) can be re-defined with the **Edit Peak** command.

The **Image Overlays** command opens the **Image Overlays** dialog box described below.

Change Profile Name is used to change the name of the currently selected lane.

Set Profile Width is used to adapt the width of the densitometer windows drawn around the lanes.

Flip Profiles changes the densitometer orientation upside down and **Expand To Edges** expands the densitometer window beyond its narrow sides over the entire length or width of the image.

If no overlay is selected, only the basic editing functions of the standard contextual menu described in the AIDA Image Analyzer Basic Concepts and Features User's Manual will be accessible.

Image Overlays

You can specify the label type and font you want to apply to the overlays (lanes and bands) using the **Image Overlays** command of the **Options** menu.

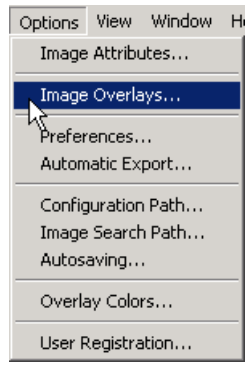


Figure 2-5 Options Menu: Opening the Image Overlays Dialog Box

After choosing **Image Overlays** from the **Options** menu, the **Image Overlays** dialog box opens allowing you to specify the labeling and marking of lanes and bands separately, using the **Lanes** and **Bands** tab respectively. In addition, you can specify marking and labeling for **RF Fronts**.

For **Lanes**, depending on the already performed evaluations, various labels can be selected by clicking the **Configure** button in the **Label** pane of the **Lanes** tab.

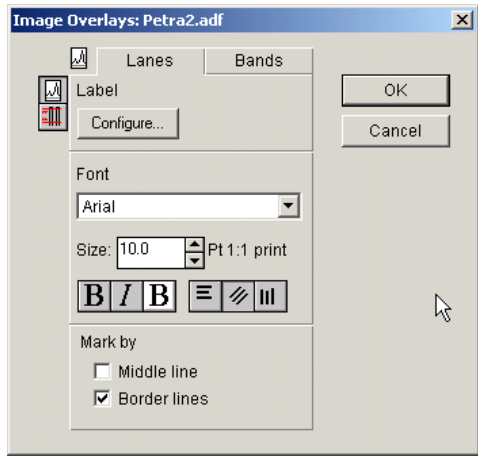


Figure 2-6 Image Overlays Dialog Box: Lanes Tab

This opens the **Columns Selection** dialog box, which provides you with a list of possible labels (e. g. lanes can be labeled by **Number**, **Profile** name or not at all.

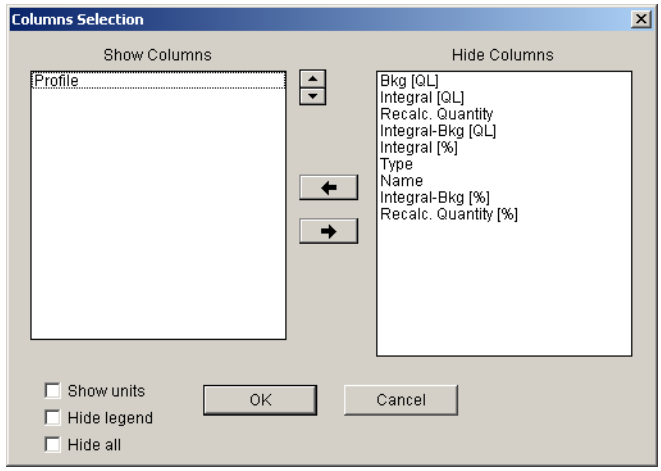


Figure 2-7 Columns Selection Dialog Box

After you have determined the type of labeling, you can set the font, font size, and font style (bold, italic, roman typeface on white background) as well as the rotation (0°, 45°, 90°) for the labels. The detected lanes can be surrounded by a frame (**Border Lines**) and tracked by a **Middle Line**.

Similar to the lanes, assigned peaks can be labeled using the **Configure** button of the **Label** pane on the **Bands** tab.

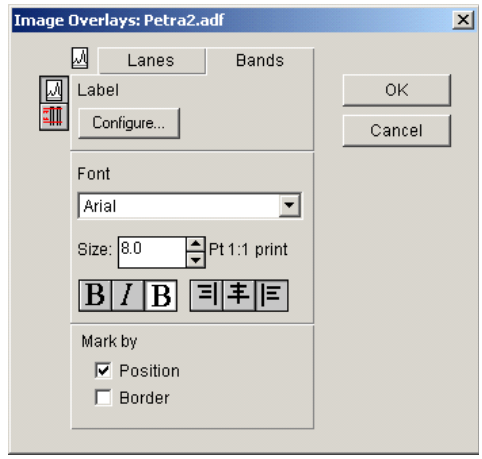


Figure 2-8 Image Overlays Dialog Box: Bands Tab

As for the lanes, use the **Font** pane of the dialog box to set the font options (font, font size, font style). Specify the position of label relative to the band marks (left, center, right) using the controls next to the font style selection buttons.

To insert marks for the peaks on the image, select the appropriate checkboxes in the **Mark By** part of the **Image Overlays** dialog box (**Position** and/or **Border**)

Besides the labeling for lanes and bands, the **Image Overlays** dialog box allows you to configure the display of RF front marks and labels:

Just click the second icon on the toolbar to switch to **1D RF Fronts** tab.

Figure 2-9 Image Overlays Dialog Box: Toolbar

To turn on the drawing of RF fronts on the image, click to select the **Draw RF Fronts** option.You can change the font size for the labels using the **Label Size** combo box. In addition, the **Location of the Corner's Labels** can be selected: The **Inner** option includes the labels within the image, whereas **Outer** puts the labels outside.

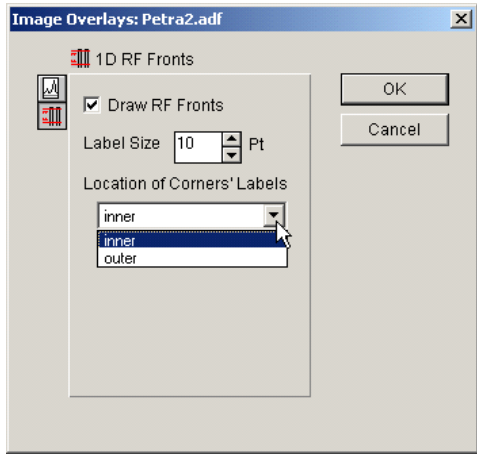
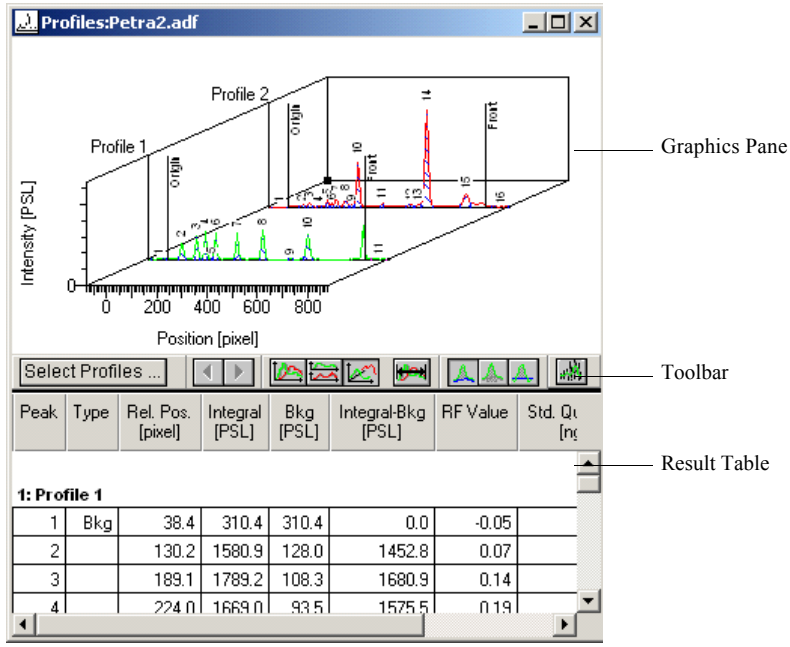


Figure 2-10 Image Overlays Dialog Box: 1D RF Fronts Tab

Profiles Window

The **Profiles** window is bipartite, the upper pane displays the change of intensity over the length of the densitometer window, integrated or averaged (as selected) over the width of the densitometer window, the lower one contains a table, listing the standards, their given and recalculated values etc.



Peak	Type	Rel. Pos. [pixel]	Integral [PSL]	Bkg [PSL]	Integral-Bkg [PSL]	RF Value	Std. Qu [ng
1	Bkg	38.4	310.4	310.4	0.0	-0.05	
2		130.2	1580.9	128.0	1452.8	0.07	
3		189.1	1789.2	108.3	1680.9	0.14	
4		224.0	1669.0	93.5	1575.5	0.19	

Figure 2-11 Profiles Window Example

In the graphics pane of the **Profiles** window, the displayed range of both axis can be zoomed:

To select a display range on an axis:

1 Choose the **Select** tool from the toolbar.

Figure 2-12 Select Tool on the Main Toolbar

2 Click at the smallest value of interest on the x-axis and drag to the biggest value of interest on the same axis with the left mouse button pressed.

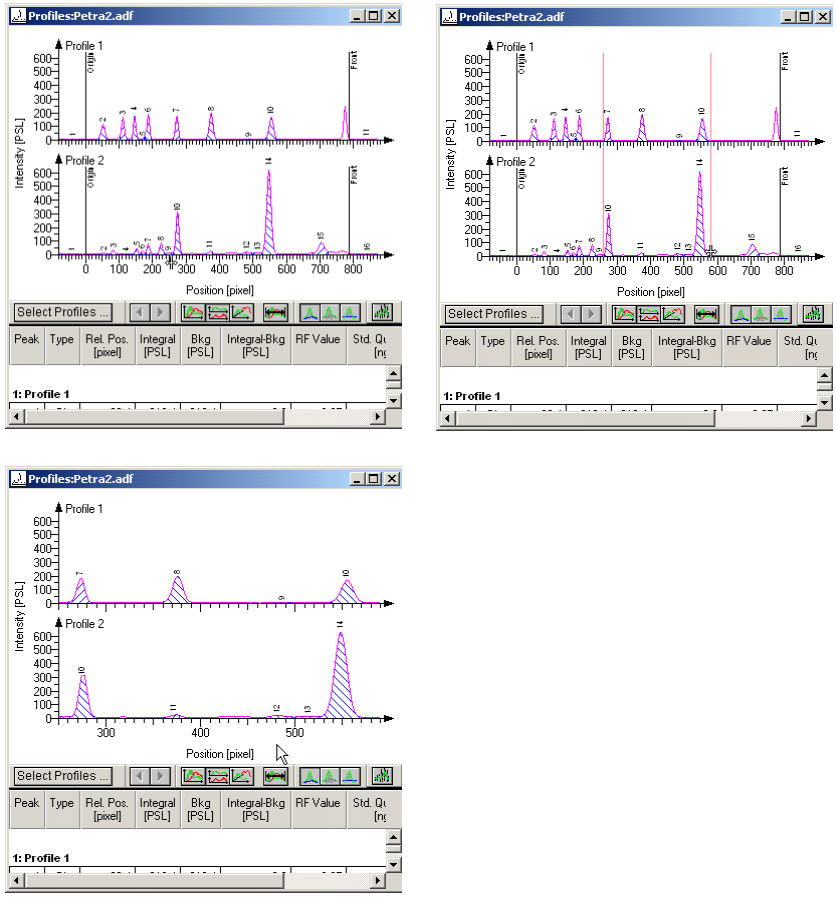


Figure 2-13 Zooming X-Axis

3 Release the mouse button.

The scaling will change accordingly.

This procedure is also applicable in 3D display mode:

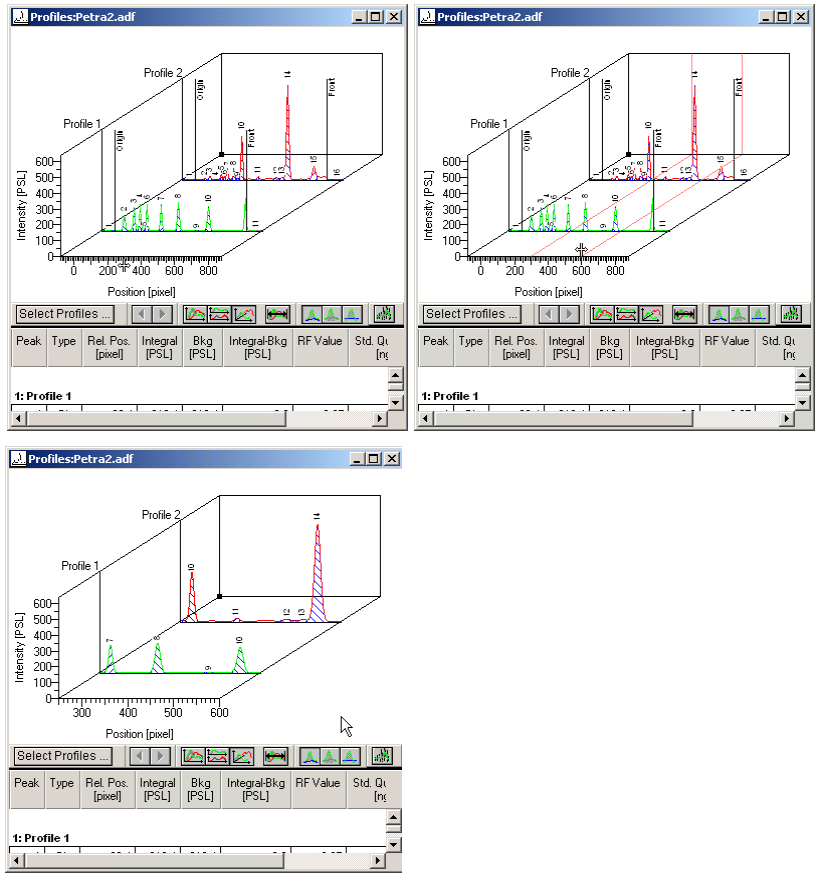


Figure 2-14 Zooming X-Axis in 3D Display Mode

To zoom out again, click the right mouse button when the ⇳ symbol is displayed. The maximal zoom-out factor is defined using the **Data Scaling** command (see below).

The same procedure can be applied to the y-axis.

To zoom into the Profiles window using the Zoom tool:

1 Click the Zoom tool on the toolbar.

Figure 2-15 Selecting Zoom Tool

2 Draw a rectangle around the area of the plot you want to see in more detail.

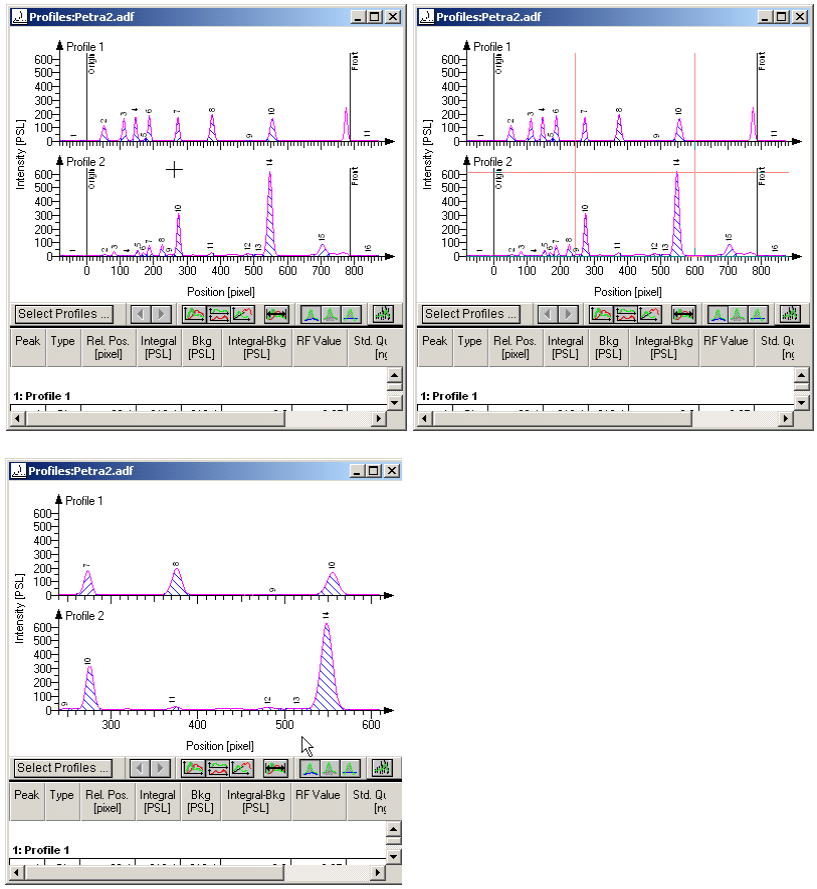


Figure 2-16 Zooming into Profiles Window

3 Release the mouse button.

4 Right-click to zoom out.

This procedure is also applicable in the 3D display mode, with the difference that you have to draw the rectangle on the back plane of the cuboid:

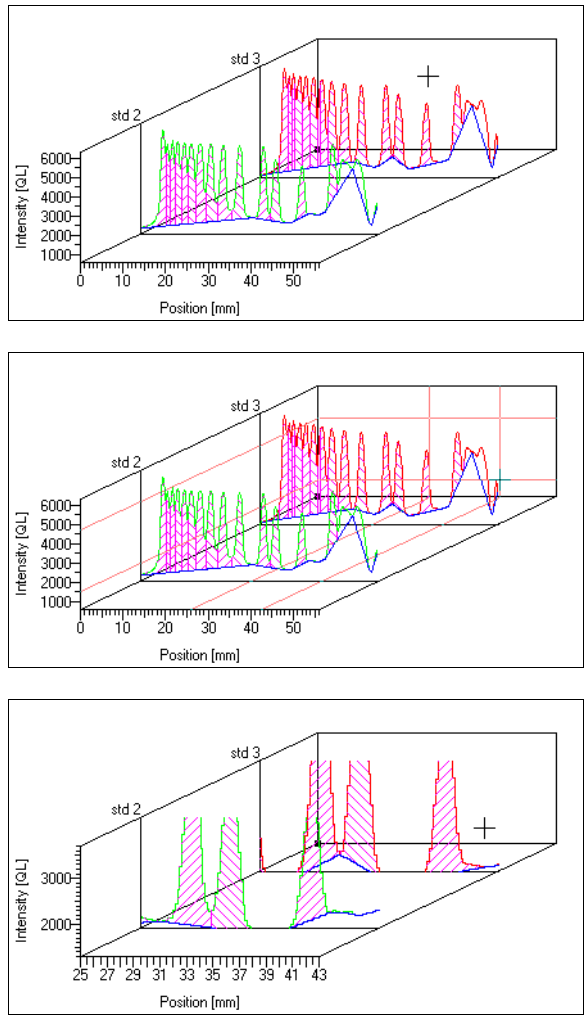


Figure 2-17 Zooming into Profiles Window in 3D Display Mode

To change your viewpoint in 3D display mode:

- Drag the axis origin with the mouse.

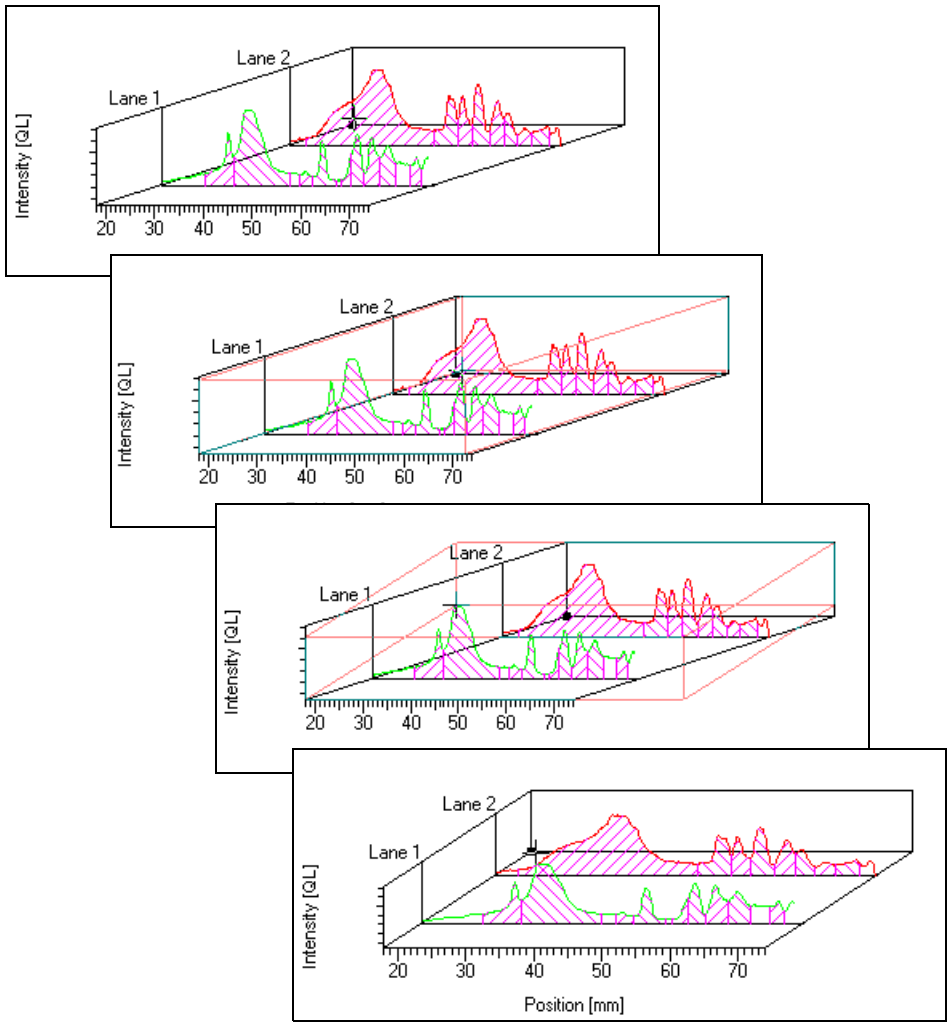


Figure 2-18 Changing 3D Viewpoint

To edit peaks:

1 Double-click the peak, whose borders or position you want to edit.
Vertical delimiter bars appear.

2 Drag the vertical delimiter bar.

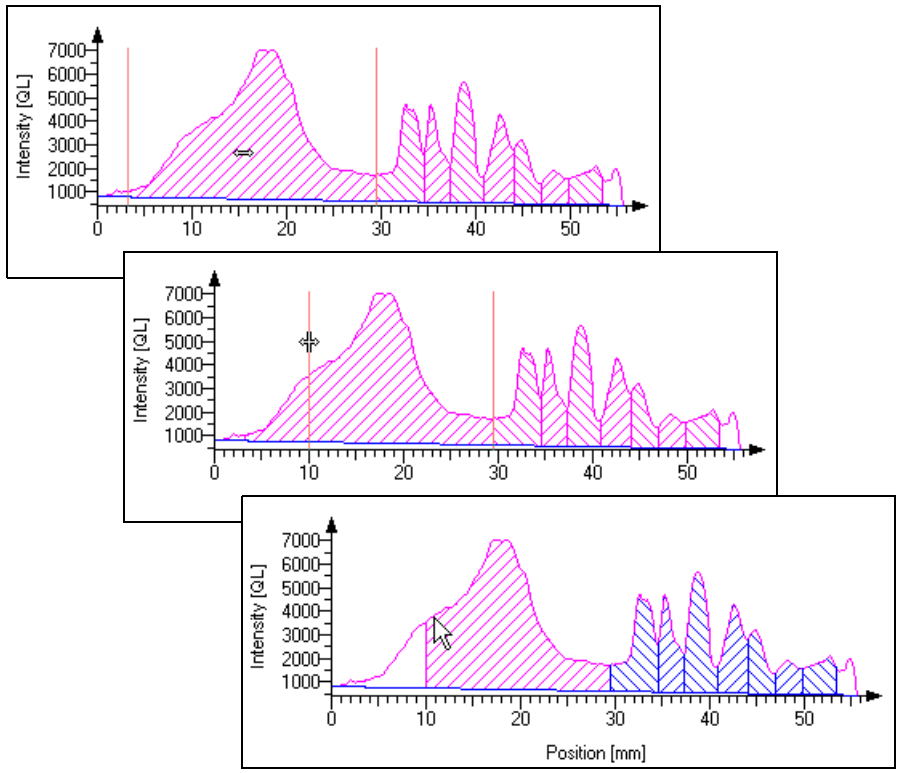


Figure 2-19 Editing Peaks

3 Right-click to confirm the changes.

To delete a peak:

1 Right-click on the hatched area indicating the peak's domain.

2 Choose **Delete Peaks** from the contextual menu.

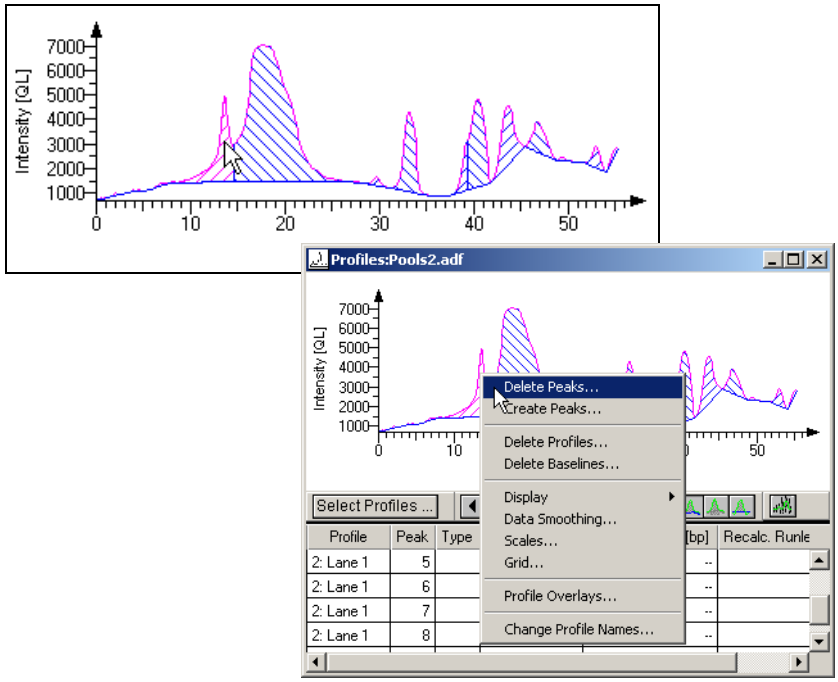


Figure 2-20 Selecting peak and Opening Contextual Menu

A dialog box opens, prompting you to confirm the command.

3 Click **OK** to delete the selected peak.

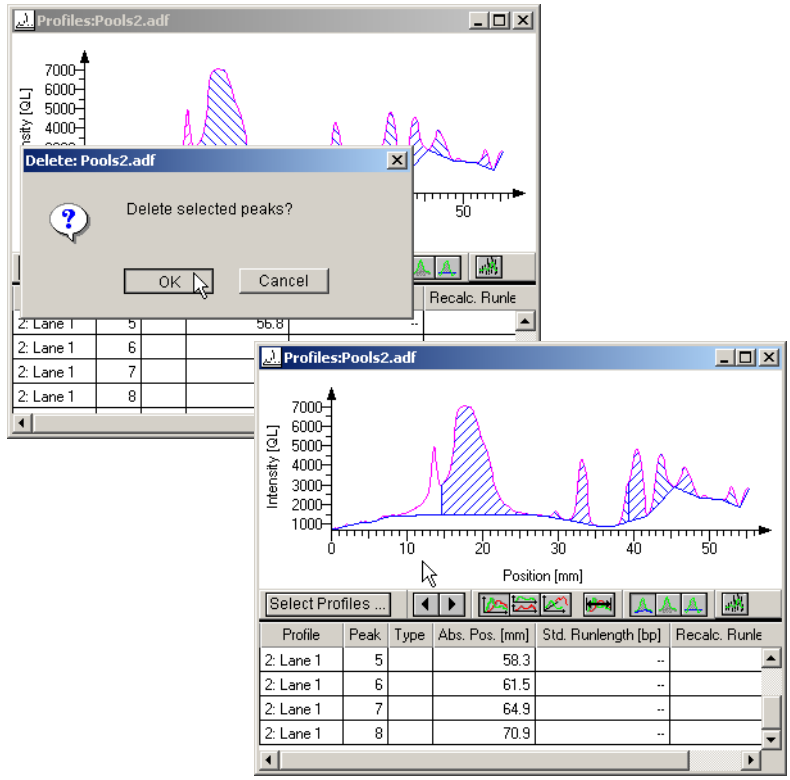


Figure 2-21 Confirming Deletion of Peak

Alternatively, you can delete peaks by first selecting the peaks in the **Image** window and pressing the DEL key on your keyboard.

Toolbar of the Profiles Window

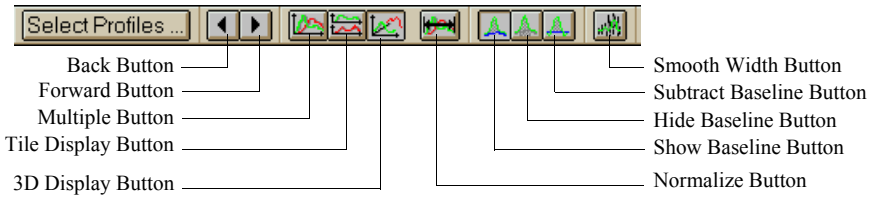


Figure 2-22 Profiles Window Toolbar

Using the **Select Profiles** button you can select any profile by its name. With the arrow buttons next to the **Select Profiles** button you can browse forward or backwards through the available profiles. The three buttons in the middle provides you with different display possibilities for the graphical output:

Multiple displays multiple curves for selected items in a single two-dimensional co-ordinate system, using different colors for each.

3D shows all curves for selected items in a single three-dimensional co-ordinate system.

Tile uses a separate 2D co-ordinate system for each curve of a selected item.

The **Normalize** button is used to normalize all displayed profiles according to the determined migration distance.

The **Show Baseline**, **Hide Baseline**, **Subtract Baseline** buttons are used to display the determined baseline, hide the baseline and subtract everything below the baseline, respectively. This function is only available if a baseline has been set in the **Band & Peak Determination** toolbox.

The **Smooth Width** button opens a dialog box to enter the smooth width for the curve display. A higher number will result in averaging over the given range of pixel, mm, inch, where the unit is set using the **Image Attributes** commands of the **Options** menu.

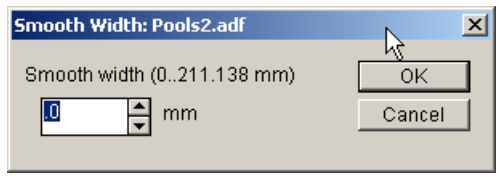


Figure 2-23 Smooth Width Dialog Box

The lower part of the **Profiles** window contains a table (henceforth *Result Table*), listing the standards, their given and recalculated values etc.

You can change the display ratio between the graphics and the table pane by dragging the delimiter line located on the bottom of the toolbar:

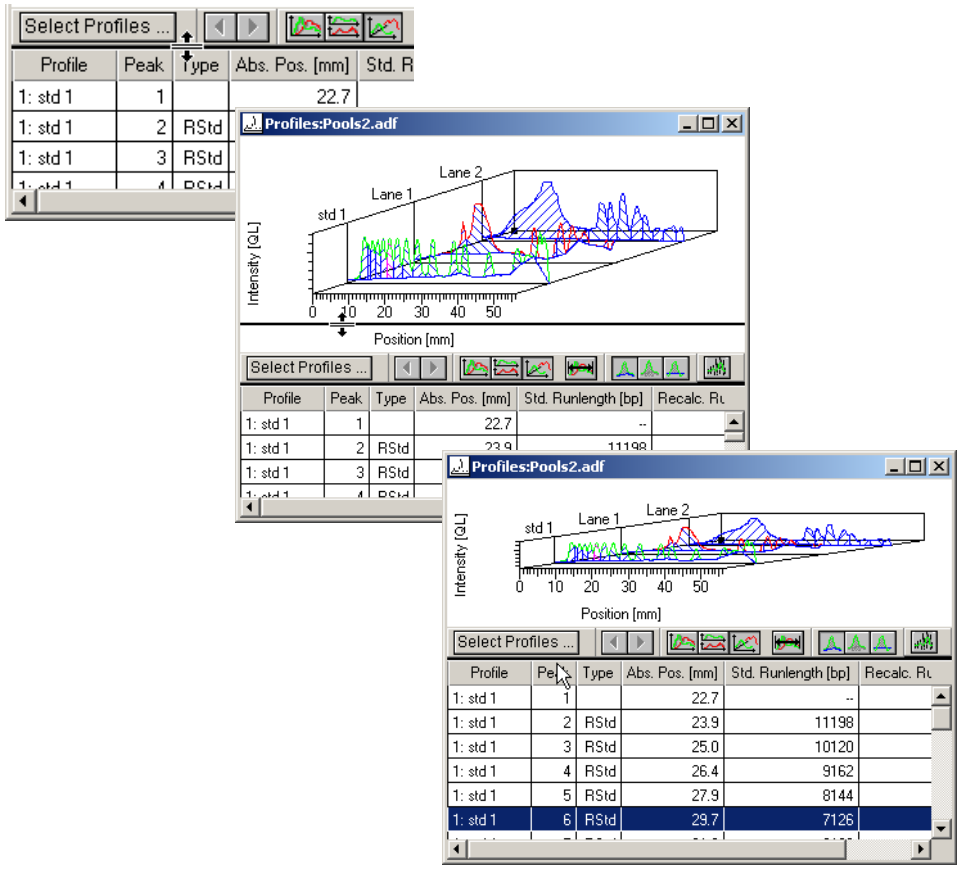


Figure 2-24 Changing the Display Ratio Between Graphics Pane And Result Table

Data Description

Available **Columns** in the **Result Table**:

Table 2-1 Columns Available in the Result Table of the Profiles Window

Column	Short Description
Profile	The index and name of the profile.
Peak	The number of the peak.
Type	The type of the peaks, e.g. QStd for Quantity Standards.
Name	The name of the peak as given by the user.
Rel. Pos. [*length unit*]	The position of the peak in the profile.
Abs. Pos. [*length unit*]	The position of the band in the image measured from the edges of the image.
Start Pos. [*length unit*]	The start position of the peak in the profile.
End Pos. [*length unit*]	The end position of the peak in the profile.
Width [*length unit*]	The width of the peak.
Height [*signal unit*]	The maximum height of the peak.
Integral [*signal unit*]	The integrated peak intensity.
Bkg [*signal unit*]	The integrated background intensity.
Bkg [%]	The percentage of the peak background in comparison to the **Sum** of all peak backgrounds, or to the value in the row which is 'Set to 100 %', if defined.
Integral-Bkg [*signal unit*]	The background-corrected integral of the peak.
Integral [%]	The percentage of the peak integral in comparison to the **Sum**., or to the value in the row which is 'Set to 100 %', if defined.
Integral-Bkg [%]	The percentage of the peak integral reduced by the background intensity in comparison to the **Sum**, or to the value in the row which is 'Set to 100 %', if defined.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit = unit of measurement, specified by the user through quantity calibration

Table 2-1 Columns Available in the Result Table of the Profiles Window

Column	Short Description
Std. Quantity [*quantity unit*]	The quantity of the peak, as entered for the calculation of the standard curve.
Recalc. Quantity [*quantity unit*]	A calculation of the peak quantity, based on the current calibration curve.
Recalc. Quantity [%]	The percentage of the peak quantity in comparison to the **Sum** of all peak quantities, or to the value in the row which is 'Set to 100 %', if defined.
RF Value	Retention factor value

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit = unit of measurement, specified by the user through quantity calibration

The following additional rows can be found or created exclusively in the **Region Report**:

Table 2-2 Additional Columns in the Region Report

Sum	Displays the scientifically correct sum for the selected column.
Ttl (Total)	Sum over the entire profile.
Remainder	Ttl – Sum

Numbers in *italics* indicate that the value was interpolated over the end of the defined standard curve and is therefore only representing a tendency, not a result. When a field is not relevant for a profile a dash [-] is displayed. The numbers can be formatted as outlined below.

You can select the number and type of columns you want to display using the contextual menu of any column in the window (see below).

To change the column order:

1. Click the heading of the column to be moved.
2. Press the mouse button and drag to the desired position in the table.

3 Release the mouse button.

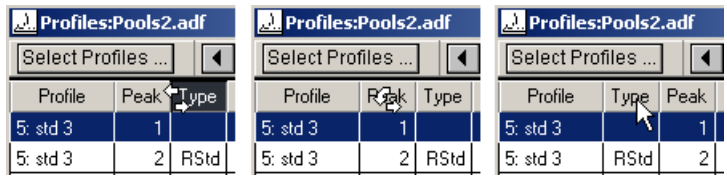


Figure 2-25 Changing Column Order

To change the column width:

1 Position the mouse pointer over the border of the column whose width you want to change.

2 Click and drag the border in the appropriate direction.

3 Release the mouse button:

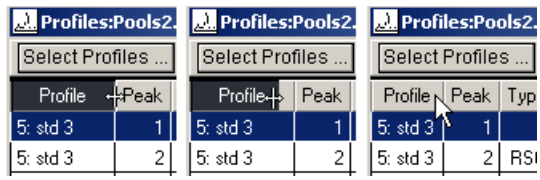


Figure 2-26 Changing Column Width

Contextual Menu of the Profiles Window (Graphics Pane)

Right-clicking on the **Profiles** window's graphics pane opens a contextual menu, providing you with commands for deleting/creating peaks, deleting profiles/baselines as well as several display settings (see below).

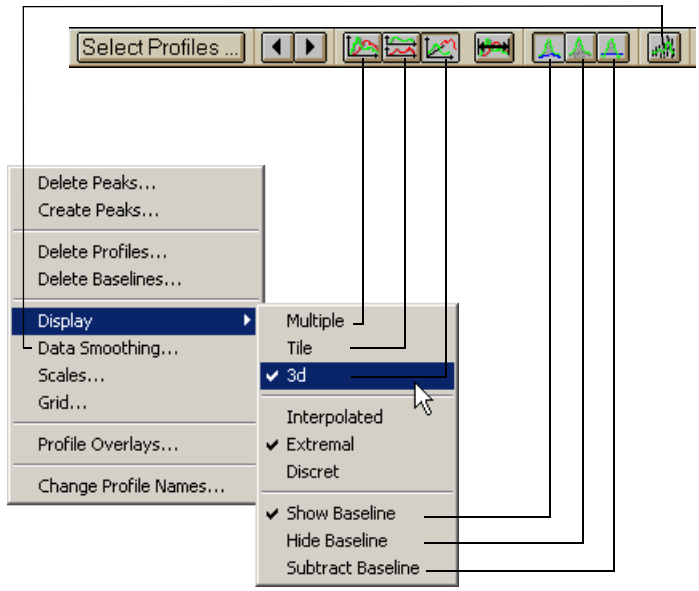


Figure 2-27 Contextual Menu of the Profiles Window's Graphics Pane

From the submenu of the **Display** command you can specify the appropriate display mode (**Multiple**, **Tile** and **3d** explained earlier) as well as how the data should be displayed: You can choose between the **Discrete** display of data points (pixels) in the profile, the display of **Extremal** data (representing the maximum value for all points between the determined data points), or the display of **Interpolated** curves between data points. Further, you can specify to show or hide the baseline using the **Show Baseline** or **Hide Baseline** command respectively. In addition you can choose to subtract the baseline from the data by selecting **Subtract Baseline**.

With the **Data Scaling** command you can configure the graphical output. The **Minimum** and **Maximum** value of the x- (**Position**) and y-axis (**Intensity**) can be selected together with a logarithmic representation for y-axis. Both axis can be set to **Automatic** scaling using the appropriate checkboxes. The **3D View Point** settings are the numerical representation of the change in 3D views as described above.

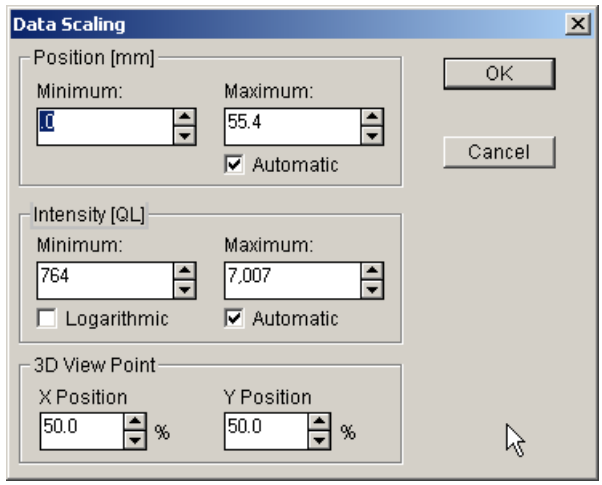


Figure 2-28 Data Scaling Dialog Box

The **Data Smoothing** function is used to smoothen the curve.

Figure 2-29 Smooth Width Dialog Box

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

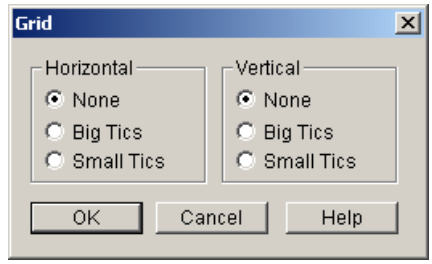


Figure 2-30 Grid Dialog Box

The **Profile Overlays** command opens a dialog box that allows you to select various labels for the peaks, together with their font, font size, font style and rotation angle. The display of peak **Area** and / or **Brackets** (indicating the bands' borders) can be selected. To turn off the marking of the peaks on the graphics display, click to deselect the **Draw Peaks** option.

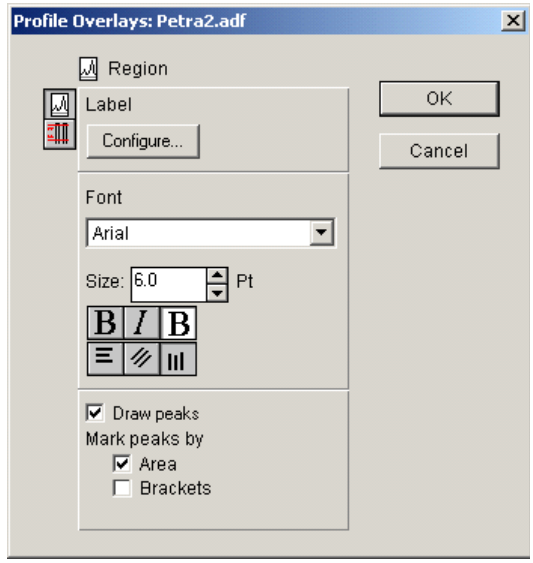


Figure 2-31 Profile Overlays Dialog Box (Label Tab)

The location of the RF fronts can be drawn into the profile by clicking the **Draw RF Fronts** option on the **1D RF Fronts** tab. Further you can specify the font size of the labels by entering a value in the **Label Size** combo box.

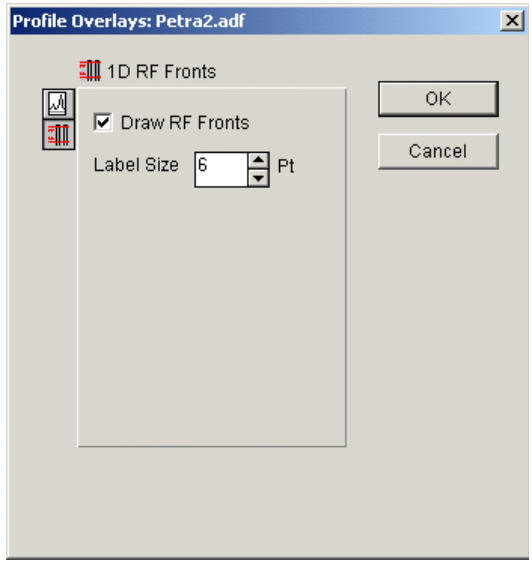


Figure 2-32 Profile Overlays Dialog Box (1D RF Fronts Tab)

With the **Change Profile Names** command you can re-name the densitometer windows and the resulting profiles according to your preferences. The names will appear in all result tables and image menus.

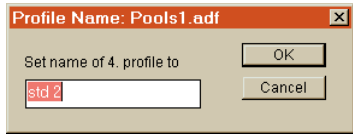


Figure 2-33 Profile Name Dialog Box

Contextual Menu of the Profiles Window (Result Table)

Each column in the **Result Table** has a contextual menu attached. To open the contextual menu, right-click on the column heading (gray area).

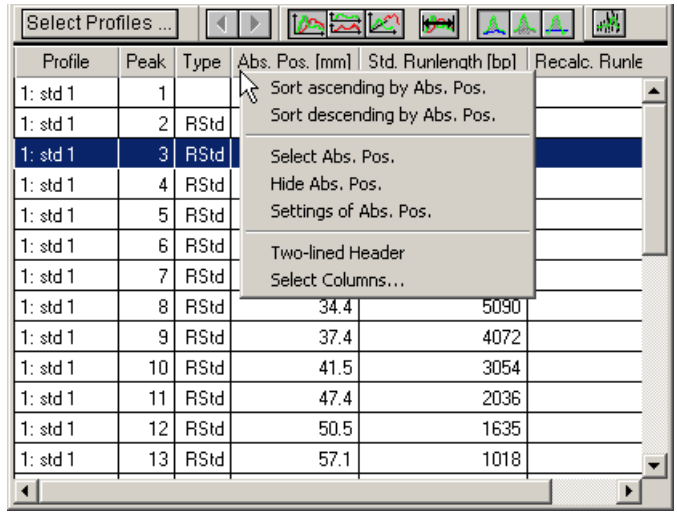

Select Profiles ...
Profile
Peak
Type
Abs. Pos. [mm]
Std. Runlength [bp]
Recalc. Runle
Sort ascending by Abs. Pos.
Sort descending by Abs. Pos.
Select Abs. Pos.
Hide Abs. Pos.
Settings of Abs. Pos.
Two-lined Header
Select Columns...
1: std 1 1
1: std 1 2 RStd
1: std 1 3 RStd
1: std 1 4 RStd
1: std 1 5 RStd
1: std 1 6 RStd
1: std 1 7 RStd
1: std 1 8 RStd 34.4 5090
1: std 1 9 RStd 37.4 4072
1: std 1 10 RStd 41.5 3054
1: std 1 11 RStd 47.4 2036
1: std 1 12 RStd 50.5 1635
1: std 1 13 RStd 57.1 1018

Figure 2-34 Contextual Menu of the Profiles Window's Result Table

With the first two commands of the contextual menu, you can sort the table of the **Result Table** by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort Ascending by**/**Sort Descending by**).

The **Select**/**Unselect** commands of the contextual menu are used to select/deselect a column to export. The column heading of selected columns are highlighted.

To hide a column, choose **Hide** command from its contextual menu. You can recover hidden columns using the **Select Columns** command (see below).

To specify the decimal digits for the value output in a column, choose **Settings of** from the contextual menu and enter the value for the decimal digits in the dialog box displayed.

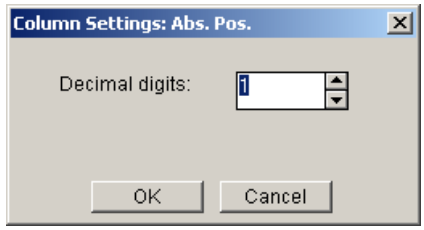


Figure 2-35 Setting Output Format for Columns

Use the **One-lined Header**/**Two-lined Header** command to reduce or expand the column heading by one line (the second line indicates the units of measurement).

To select/deselect the columns that should be displayed in the Result Table:

1. Choose **Select Columns**.

   A dialog box appears displaying two lists (**Show Columns**/**Hide Columns**).

2. Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists.

3. To deselect a particular item, just click it again.

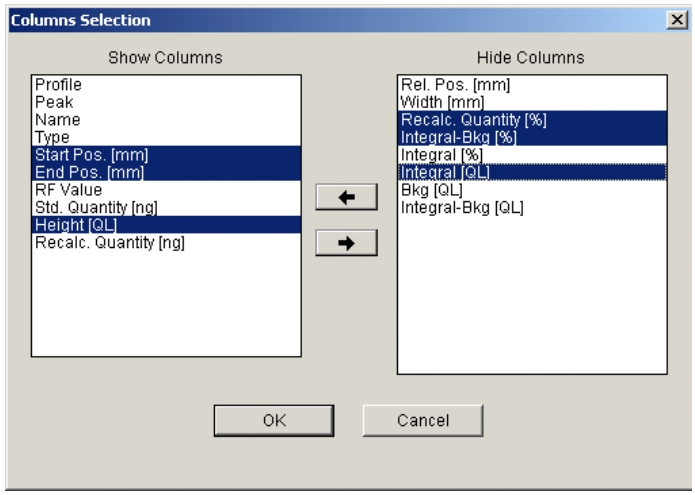


Figure 2-36 Columns Selection Dialog Box

In addition, each row of the table has a contextual menu attached, which includes commands for displaying special row types like **Sum**, **Total**, **Remainder** as well as for changing profile names.

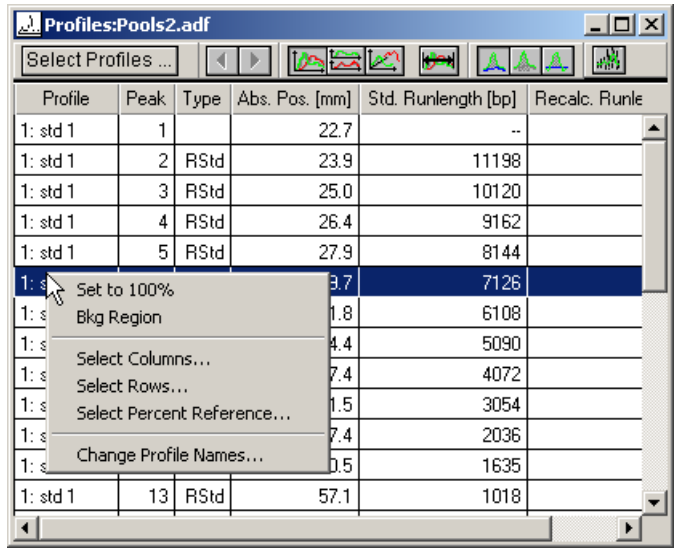


Figure 2-37 Contextual Menu of a Result Table Row

Set to 100 % will set the value in the active row to 100 % instead of using the **Sum** value as 100% and recalculate the other values accordingly.

To select the columns that should be displayed in the Result Table, choose the **Select Columns** command (see above).

Using the **Select Rows** command, you can choose four special row types to be displayed in the Result Table. In the dialog box that appears, click to select or deselect the appropriate options. Clicking **Total**, **Sum**, and/or **Remainder** inserts the respective row(s) into the table. **Separator** displays an extra separator row showing the name of the profile.

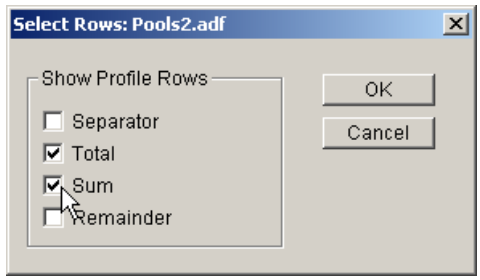


Figure 2-38 Select Rows Dialog Box

Select Percent Reference: This command allows you to set the reference value for the percentage calculation. This can be either a distinguished peak defined by the **Set to 100 %** command of the table contextual menu (see above), the value for the total profile, or the **Sum**. The later two are set via the following dialog box.

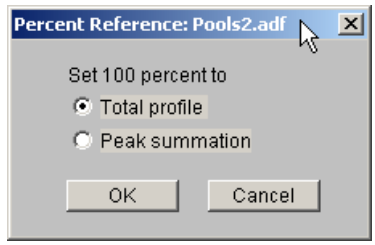


Figure 2-39 Percent Reference Dialog Box

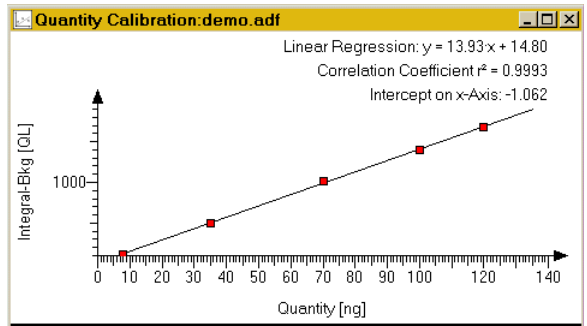
The **Change Profile Names** command allows to re-name the densitometer windows and resulting profiles according to your preferences. The names will appear in all result tables and image menus.

Figure 2-40 Profile Name Dialog Box

Quantity Calibration Window

To open the Quantity Calibration window:

- Choose **Quantity Calibration** from the **View** menu, *or*
- Double-click the miniaturized calibration curve display on the **Quantity Calibration** toolbox.


Quantity Calibration:demo.adf
Linear Regression: y = 13.93·x + 14.80
Correlation Coefficient r² = 0.9993
Intercept on x-Axis: -1.062
Integral-Bkg [QL]
1000
0 10 20 30 40 50 60 70 80 90 100 120 140
Quantity [ng]

Profile	Peak	Integral-Bkg [QL]	Quantity Std. [ng]	Quantity Recalc. [ng]
1: Standa	9	1675.8	120.0	119.25
1: Standa	11	1402.9	100.0	99.66
1: Standa	13	1019.2	70.0	72.11
11: Profile	1	494.6	35.0	34.44
11: Profile	2	119.9	8.0	7.54

Figure 2-41 Quantity Calibration Window Example

The **Quantity Calibration** window is bipartite, the upper pane displays the curve of the calibration function, the underlying function, the correlation coefficient and the x-axis intercept. The lower one contains a table, listing the standards, their given and recalculated standard values, so you can easily evaluate their differences. This can be useful to estimate the error for a certain amount calculated for an unknown band.

The graphical representation can be adapted using the contextual menu attached. For a more detailed view of the plot, you can activate the AIDA standard zoom functions described above.

The table can be exported as a text file or copied into the clipboard.

Additional columns can be displayed. Just right-click on the table heading and choose the **Select Columns** command from the contextual menu, that appears. The **Columns Selection** dialog box opens, allowing you to select the desired columns from the list.

Contextual Menu of the Quantity Calibration Window

The contextual menu of the upper pane of the **Quantity Calibration** window contains two commands: **Scales** and **Grid**.

With the **Scales** command you can configure the graphical output. After clicking the **Scales** command, the **Data Scaling** dialog box opens. Here the **Minimum** and **Maximum** value of the x- (Concentration) and y-axis (Intensity) can be selected. In addition, you can turn on logarithmic representation and automatic scaling of either axis separately by clicking the appropriate option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

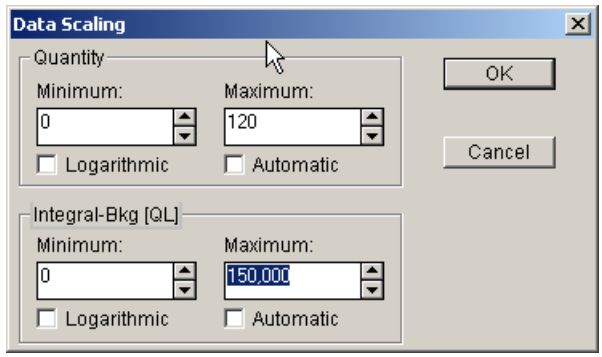


Figure 2-42 Data Scaling Dialog Box (Quantity Calibration)

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

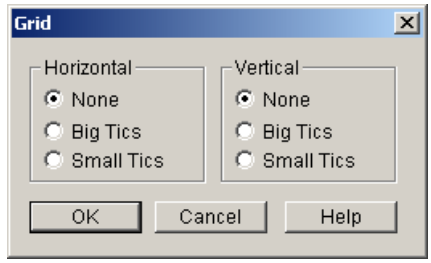


Figure 2-43 Grid Dialog Box

The table in the lower part of the **Quantity Calibration** window provides its own contextual menu:

The column headings are equipped with the standard contextual menus described in the “Profiles Window” section of this manual. If you right-click on any of the table rows, the row's contextual menu opens, allowing you to **Select Columns** for displaying or **Delete Standards** from the table.

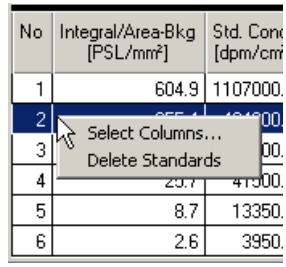


Figure 2-44 Quantity Calibration Window (Row Contextual Menu)

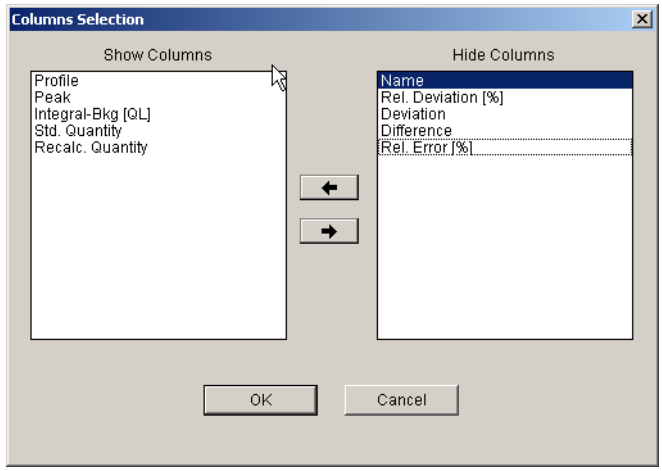


Figure 2-45 Selecting Columns to be Displayed

The following columns are available:

Table 2-3 Columns Available in the Quantity Calibration Window

Columns	Short Description
Profile	The index and name of the profile.
Peak	The number of the peak in the profile.
Name	The name for the peaks as given by the user.
Integral-Bkg [*signal unit*]	The background-corrected integral of the peak.
Std. Quantity [*quantity unit*]	The quantity of the peak, as entered for the calculation of the quantity standard curve.
Recalc. Quantity [*quantity unit*]	A calculation of the peak quantity, based on the current quantity calibration curve.
Difference [*unit*]	The difference without sign between the entered standard and the recalculated value.
Rel. Error [%]	The relative difference without sign of the entered standard and the recalculated value compared to the standard value (= Difference / Std. Quantity).
Deviation [*unit*]	The difference with sign between the entered standard and the recalculated value (Recalc. Quantity – Std. Quantity).
Rel. Deviation [%]	The relative difference with sign of the entered standard and the recalculated value compared to the standard value (= Deviation / Std. Quantity).

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit = unit of measurement, specified by the user through quantity calibration

Audit Trail Window

In the **Audit Trail** window you can view all GLP relevant changes to the currently active evaluation (as defined via the **Data Trail Settings for** controls of the **GLP Supporting Tools** toolbox), including timestamp, user-id, evaluation status and reasons for evaluation changes indicated by the current user.

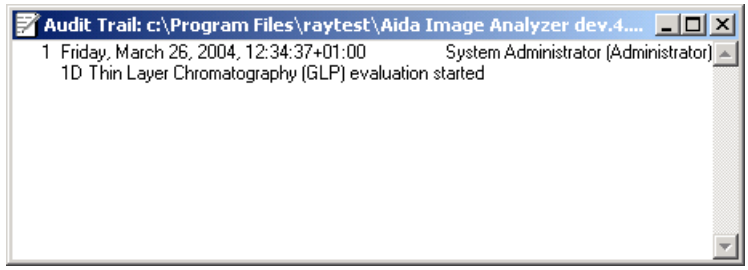

Audit Trail: c:\Program Files\raytest\Aida Image Analyzer dev.4....
1 Friday, March 26, 2004, 12:34:37+01:00 System Administrator (Administrator)
1D Thin Layer Chromatography (GLP) evaluation started

Figure 2-46 Audit Trail Window

To open the **Audit Trail** window, either choose the **Audit Trail** command from the **View** menu, or alternatively click the **View Audit Trail** button in the **Data Trailing** pane of the **GLP Supporting Tools** toolbox.

Additional Toolboxes in 1D TLC

In addition to the standard AIDA toolboxes **Display Control** and **Publishing Layout**, the following toolboxes become available after selecting the **1D TLC** mode.

- TLC Calibration
- Lane & Peak Determination
- Quantity Calibration
- GLP Supporting Tool

You can access these additional toolboxes from the **Evaluation** menu or by clicking the appropriate button on the toolbar.

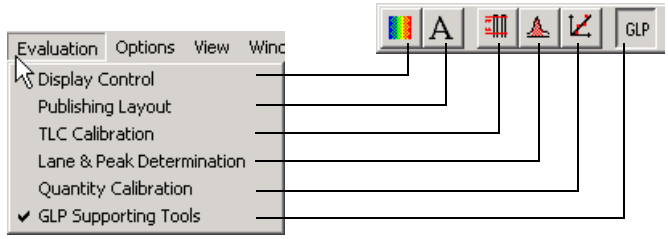


Figure 2-47 Mapping between Evaluation Menu Entries and Toolbar Buttons

1D TLC Calibration Toolbox

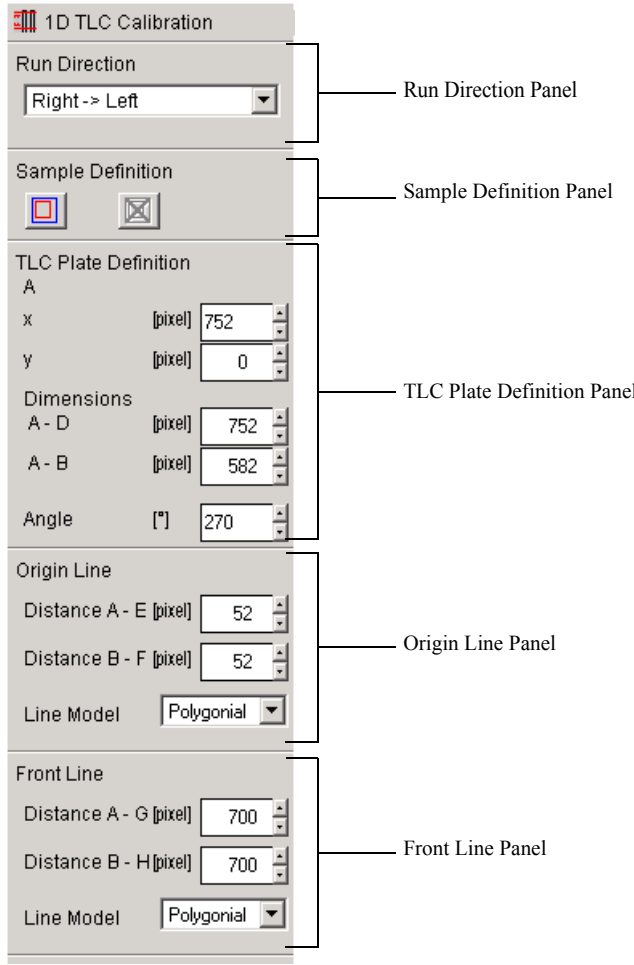
The **1D TLC Calibration** toolbox is used to set the TLC plate and the origin/front overlays.

To display the 1D Calibration toolbox:

- On the main menu, click **Evaluation** and choose the **1D TLC Calibration** entry.

  or

- Click the **1D TLC Calibration** button on the toolbar.



Run Direction Panel

From the **Run Direction** pull-down menu you can select the run directions used to develop the TLC plate from which the image was obtained. There are two vertical (**Top -> Bottom** and **Bottom -> Top**) and two horizontal options (**Right -> Left** and **Left -> Right**) available.

Sample Definition Panel

Use the two buttons of the **Sample Defintion** panel to create or delete the TLC plate overlay.

Figure 2-48 Sample Definition Panel

TLC Plate Definition Panel

The various combo boxes of the **TLC Plate Definition** panel allow you to set the position of the TLC plate overlay by entering the appropriate values for the **Position of Corner A** (the upper right corner of the plate) and the horizontal (**Distance A - B**) and vertical **Dimensions** (**Distance A - D**) of the plate together with its **Angle**.

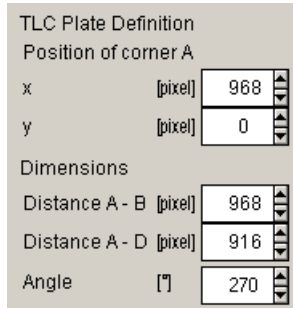


Figure 2-49 TLC Plate Definition Panel

Origin Line Panel

The **Origin Line** panel of the toolbox provides you with options to determine the position and **Line Model** of the origin line. Enter the appropriate values for the origin (**Distance A - E** and **Distance B - F**) of the plate as well as the **Line Model** (**Polygonial**, **Circular**, **Parabolic**).

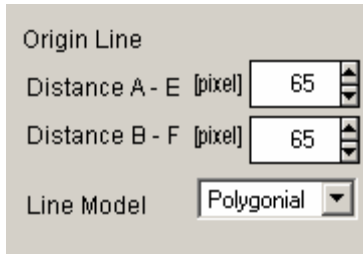


Figure 2-50 Origin Line Panel

Front Line Panel

The **Front Line** panel of the toolbox provides you with options to determine the position and **Line Model** of the front line. Enter the appropriate values for the front (**Distance A - G** and **Distance B - H**) of the plate as well as the **Line Model** (**Polygonial**, **Circular**, **Parabolic**).

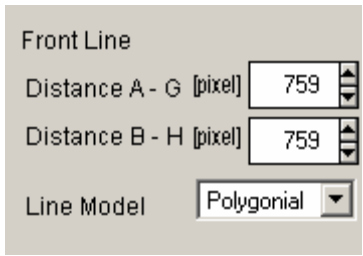


Figure 2-51 Front Line Panel

The units of measurement can be changed via the **Image Attributes** option of the **Options** menu.

Lane & Peak Determination Toolbox

The controls of the **Lane & Peak Determination** toolbox allow you to define lanes (profiles) and peaks on the image for signal intensity evaluation.

To display the Lane & Peak Determination toolbox:

• On the main menu, click **Evaluation** and choose **Lane & Peak Determination**.

Alternatively you can click the **Lane & Peak Determination** button on the toolbar.

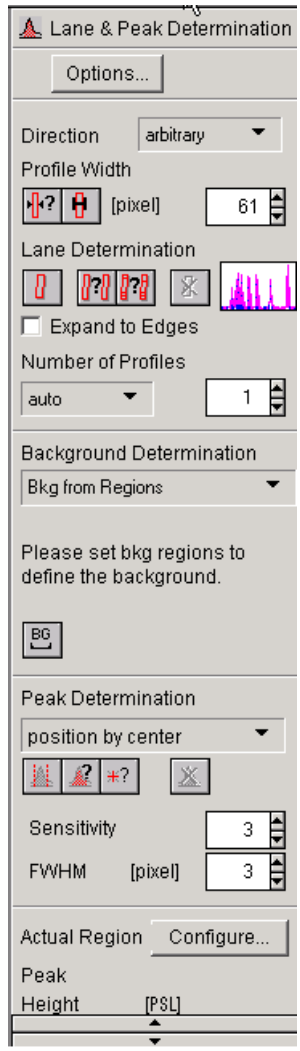


Figure 2-52 Lane & Peak Determination toolbox

Clicking the **Options** button changes the content of the toolbox, revealing options for the lane as well as the peak determination:

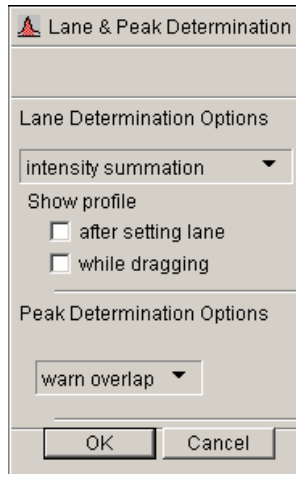


Figure 2-53 Lane & Peak Determination Options Panel

A profile can represent the **Intensity Summation**, the **Averaged Intensity** or the Optical Density. Select the appropriate option from the pull-down menu of the **Lane Determination Options** section. For applications with radioactive TLC, usually **Intensity Summation** is used.

If the **After Setting Lane** option is checked, the **Profiles** window opens directly after the densitometer window has been drawn .

If the **While Dragging** option is checked, the profile of the currently dragged densitometer window is continuously updated in the miniaturized profile window of the toolbox.

The **Peak Determination Options** pull-down menu is used to define AIDA's action if an overlap of peak regions is detected:

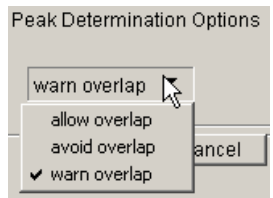


Figure 2-54 Peak Determination Options Pull-Down Menu

Allow Overlap, **Avoid Overlap** and **Warn Overlap** can be selected. **Avoid Overlap** will disable the overlapping of regions by either keeping peaks separate or by unifying two strongly overlapping peaks to one peak, after the region is confirmed.

After setting all necessary options click **OK** to return to the original toolbox content.

Densitometer windows can be drawn **Arbitrary** (in any angle), **Horizontal**, **Vertical**, or in **Run Direction**. Select the appropriate direction from the **Direction** pull-down menu.

The controls of the **Profile Width** section are used to automatically or manually determine the lane width:

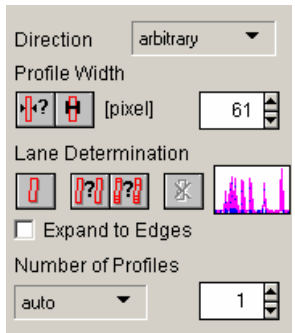


Figure 2-55 Profile/Profile Width/Lane Determination Panel

Select this button to enable automatic lane width measurement.

This button is used to determine the lane width manually.

Clicking this button activates the manual lane setting tool.

These two buttons activate the automatic lane detection tool with different options. If the left button is selected, only lanes will be searched. Clicking the right button initiates the combined detection of lanes and peaks.

Use this button to delete a selected profile.

If the **Expand to Edges** option is checked, the lanes that are set by using the manual lane setting tool or the automatic lane detection tool will be expanded to the edges of the TLC plate overlay, if one is set, or to the edges of the image if no TLC plate overlay is set.

The miniaturized **Profiles** window on the far left side of the **Lane Determination** section displays the currently selected profiles of the densitometer window.

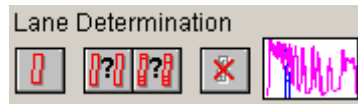


Figure 2-56 Lane Determination Section

The **Number of Profiles** is used only for the automatic lane detection tool. If **Fixed** is selected, the automatic lane detector assumes that the maximal number of profiles to be found is equal to the value selected from the combo box on the right of the **Number of Profiles** pull-down menu.

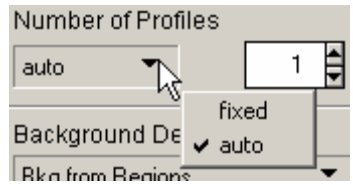


Figure 2-57 Number of Profiles Pull-Down Menu

If **Auto** is selected, the number is ignored and the automatic lane detector tries to find out the number of profiles itself.

Background Determination Section

Perform background determination if you want to quantify bands after standardization. There are two methods for determining the background: the first one (**Bkg from Regions**) determines the background from regions set by the user in the **Image** or **Profiles** window, the second one (**Bkg from Baseline**) uses a baseline set by the user in the **Profiles** window.

To set the base for the background determination, choose one of the options provided by the **Background Determination** pull-down menu.

Figure 2-58 Background Determination Pull-Down Menu

Selecting **Bkg from Regions** allows you to set regions as background by clicking this button.

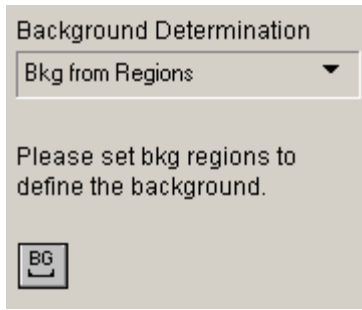


Figure 2-59 Bakground Determination Section

Choosing **Bkg from Baseline** from the **Background Determination** pull-down menu changes the content of the **Background Determination** section to reveal the baseline determination controls.

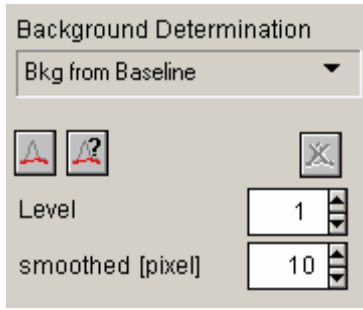


Figure 2-60 Baseline Determination Controls

Clicking this button activates the **Edit Baseline** function.

This button removes the baseline(s) of the currently selected profile(s).

With this button the baseline can be determined automatically. Use the **Smoothed** parameter to omit single pixel noise. The **Level** of sensitivity can be set to various values reaching from 0 – insensitive to 10 – extremely sensitive.

Peak Determination Section

This part of the toolbox is used for the determination of peaks.

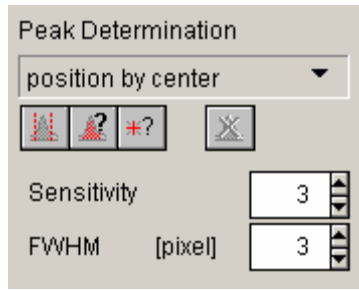


Figure 2-61 Peak Determination Section

Use the pull-down menu to mark the position of the band by **Position by Maximum** or **Position by Center**.

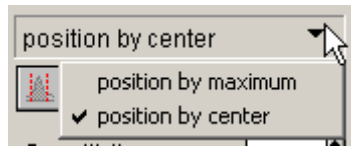


Figure 2-62 Peak Determination Pull-Down Menu

This button is used for manual peak assignment.

Click this button to delete all currently selected peaks.

To search peaks automatically within the active profile(s) this button should be clicked after choosing the appropriate **Sensitivity** and **FWHM** parameters.

Use this button to search for individual bands by clicking on them.

Actual Region Section

The **Actual Region** section provides information about the selected region. Use the **Configure...** button to add or delete categories from the list shown below the **Configure...** button. This opens the **Column Selection** dialog box described in the Profiles Window section of this manual.

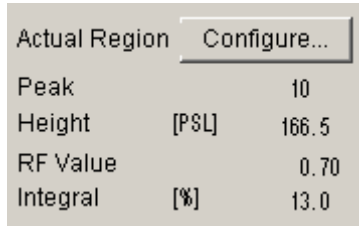


Figure 2-63 Actual Region Section

Quantity Calibration Toolbox

The controls of the **Quantity Calibration** toolbox allow you to perform quantity calibrations of 1D densitometers by assigning amount standards to the bands.

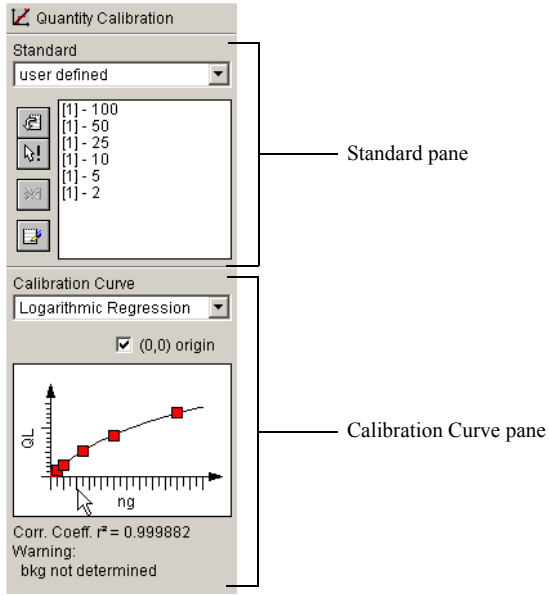


Figure 2-64 Quantity Calibration Toolbox (Overview)

To display the Quantity Calibration toolbox:

- On the main menu, click **Evaluation**, and then click **Quantity Calibration**.

Alternatively you can select the **Quantity Calibration** button on the toolbar.

Standard Pane

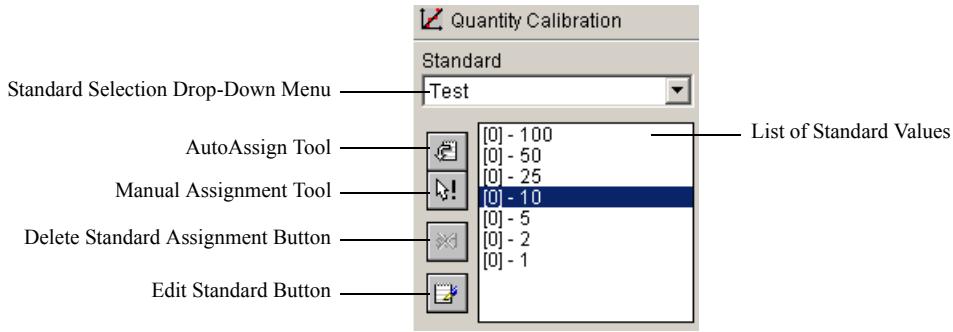


Figure 2-65 Standard Pane (Overview)

Manual Assignment Tool: This button allows you to assign amount standards manually to the bands by entering appropriate values via a dialog box.

AutoAssign Tool: Use this button to semi-automatically apply Standard values to bands one by one using a predefined list. The predefined list can be selected from the **Standard Selection** drop-down menu. The specified standard values are displayed in the **List of Standard Values** pane below the drop-down menu.

Delete Standard Assignment Button: Using this button you can delete selected standard assignment(s).

Edit Standard Button: Use this button to enter and edit predefined standards. Clicking this button opens the **Quantity Standards** dialog box.

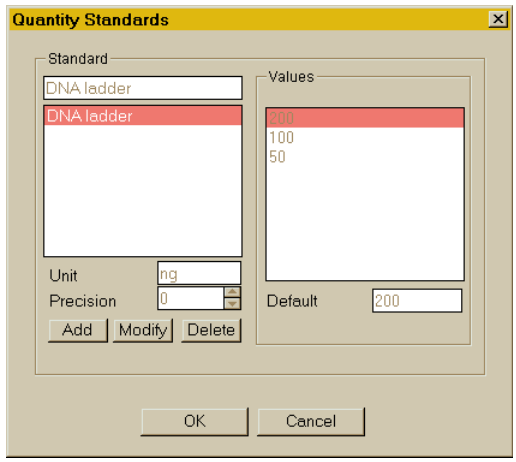


Figure 2-66 Quantity Standards Dialog Box

In the **Quantity Standards** dialog box the **Standard** name and **Values** are entered and the dimension of the amounts can be selected.

The **Add**, **Modify** and **Delete** buttons are used to alter the list of standards.

All standards defined should be saved for future use. This is also true for one-time standards because evaluation get frequently re-done, which means that the standard values are lost unless entered in the list.

Calibration Curve Pane

In the **Calibration Curve** pane of the **Quantity Calibration** toolbox the mathematical calculation of the standard curve can be selected and the result is displayed in the graph at the lower end of the toolbox.

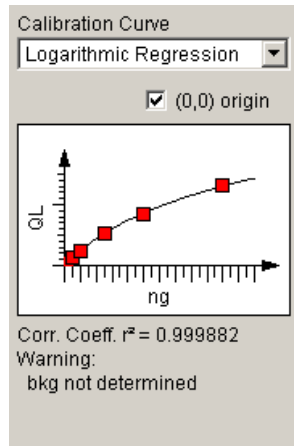


Figure 2-67 Calibration Curve Pane

The **Calibration Curve** drop-down menu provides the following functions:

- Linear Interpolation
- Linear Regression
- Logarithmic Regression
- Log. Weighted Regression

Linear Interpolation calculates lines to join each data point.

Linear Regression optimizes a linear graph so that the deviation of the actual points from the curve is minimized.

Logarithmic Regression is used to determine the standard curve with logarithmic characteristics, which is found in many saturating effects (e.g. absorption).

Log. Weighted Regression can be described as linear regression using a double logarithmic scaling. The result is a better optimization of the curve fit for the smaller values, instead of a better fit for the higher values (**Linear Regression**).

Click to select the **Origin** option to force the curve through the origin.

The correlation coefficient (**Corr. Coeff.**) of the estimation is shown below the curve. A value of 1 identifies a perfect correlation, a value of 0 indicates full independence of values. Good standard curves should have value very near to 1.

Double-clicking on the graphics pane opens the **Quantity Calibration** window (see the "Quantity Calibration Window" section of this manual for details).

Sometimes a warning will be displayed below the graphics pane, stating 'bkg not dertermined', to indicate that no background region has been assigned yet. It can also indicate the lack of a local background for some regions.

GLP Supporting Tools Toolbox

The **GLP Supporting Tools** toolbox is used to specify all GLP relevant settings.

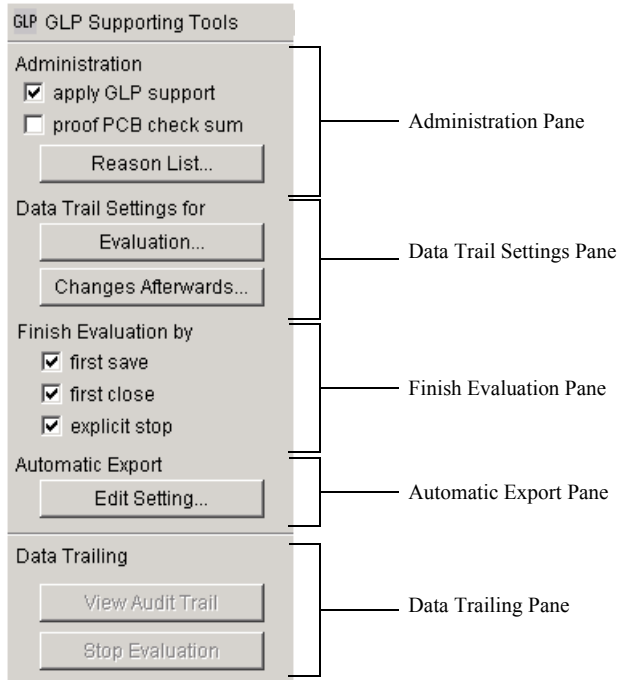


Figure 2-68 GLP Supporting Tools Toolbox (Overview)

To display the GLP Supporting Tools toolbox:

- On the main menu, click **Evaluation**, and then click **GLP Supporting Tools**,

  or

- Select the **GLP Supporting Tools** button on the toolbar.

Note – To configure the GLP support, administrator rights are required. Therefore make sure that you are logged in as system **Administrator** to change the GLP settings. If you are logged in as normal user, the **GLP Supporting Tools** toolbox is considerably reduced, providing you with the **Data Trailing** controls only.

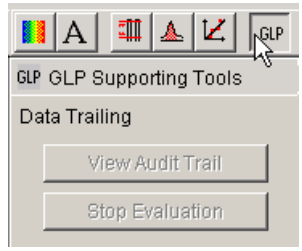


Figure 2-69 GLP Supporting Tools Toolbox (User Account)

Administration Pane

In the **Administration** pane of the **GLP Supporting Tools** toolbox you can configure all GLP support settings.

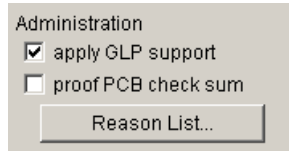


Figure 2-70 Administration Pane (Initial State)

Since this is only possible, if no evaluation has been started, ensure that no image is loaded into AIDA yet.

To enable GLP support:

- Click the **Apply GLP Support** option.

The **Proof PCB Checksum** option enables the PCB image authentification feature.

Note – Older PCB images do not incorporate checksums. So if you want to apply the **GLP Supporting Tools** controls to those images, make sure that this option is disabled.

The **Reason List** button opens the **Audit Trail Reason List** dialog box for predefining evaluation change reasons.

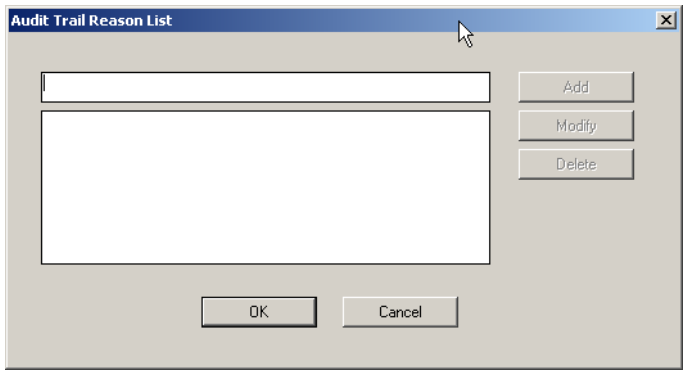


Figure 2-71 Audit Trail Reason List Dialog Box

Data Trail Settings Pane

The behavior of the GLP recording function is configured using the **Data Trail Settings for** controls:

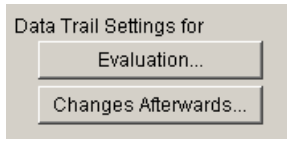


Figure 2-72 Data Trail Settings Pane

1 Click the **Evaluation** button to set the recording options for changes during evaluation.

The **Audit Trail Configuration: Evaluation** dialog box appears.

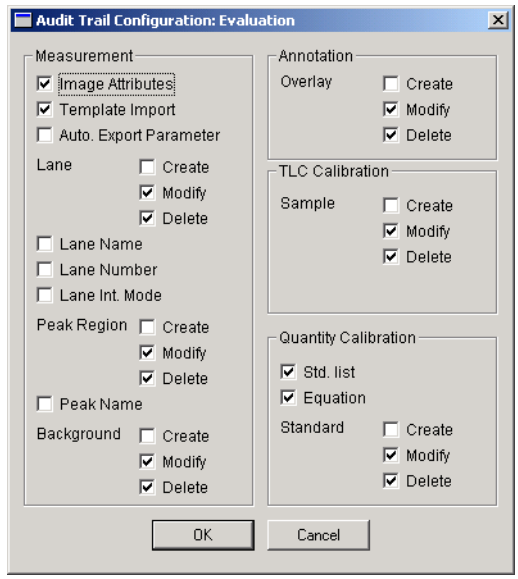


Figure 2-73 Audit Trail Configuration: Evaluation

2 Specify which user actions should be recorded.

This includes for example the modification/deletion of annotations, changing calibration method and/or calibration curve or modifying/deleting regions.

If the working operator performs one of the actions checked in the **Audit Trail Configuration** dialog box, a dialog box appears, prompting the user to give reasons for the desired change.

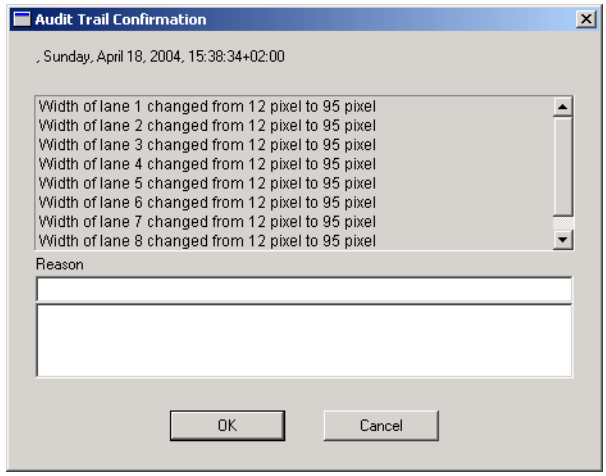

Audit Trail Confirmation
, Sunday, April 18, 2004, 15:38:34+02:00
Width of lane 1 changed from 12 pixel to 95 pixel
Width of lane 2 changed from 12 pixel to 95 pixel
Width of lane 3 changed from 12 pixel to 95 pixel
Width of lane 4 changed from 12 pixel to 95 pixel
Width of lane 5 changed from 12 pixel to 95 pixel
Width of lane 6 changed from 12 pixel to 95 pixel
Width of lane 7 changed from 12 pixel to 95 pixel
Width of lane 8 changed from 12 pixel to 95 pixel
Reason
OK
Cancel

Figure 2-74 Audit Trail Confirmation Dialog Box

The desired modification is blocked until the user confirms the action by entering the intention for the change explicitly in the **Audit Trail Confirmation** dialog box.

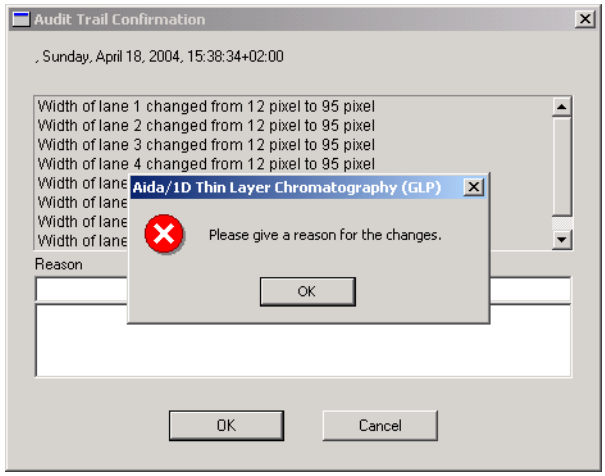

Audit Trail Confirmation
, Sunday, April 18, 2004, 15:38:34+02:00
Width of lane 1 changed from 12 pixel to 95 pixel
Width of lane 2 changed from 12 pixel to 95 pixel
Width of lane 3 changed from 12 pixel to 95 pixel
Width of lane 4 changed from 12 pixel to 95 pixel
Aida/1D Thin Layer Chromatography (GLP)
Please give a reason for the changes.
OK
Reason
OK
Cancel

Figure 2-75 GLP Error Message Box

As for changes during evaluation you can also set recording options for changes afterwards. Just click the **Changes Afterwards** button on the **Data Trail Settings for** pane. The **Audit Trail Configuration: Changes Afterwards** dialog box appears, allowing you to select the actions to be watched by the GLP system.

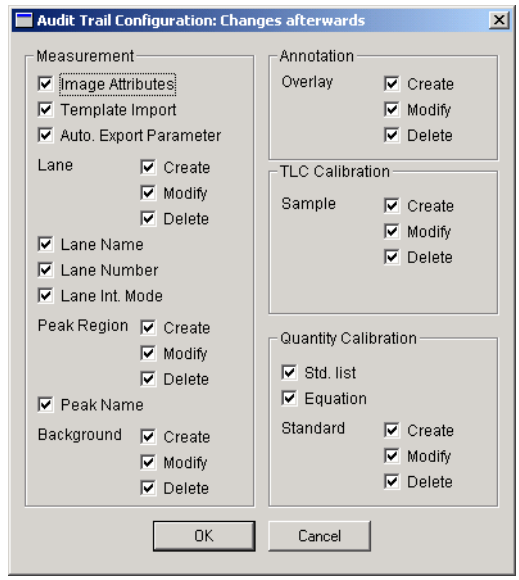


Figure 2-76 Audit Trail Configuration: Changes Afterwards Dialog Box

Finish Evaluation Pane

The checkboxes of the **Finish Evaluation by** pane allow you to specify the user action which indicates the end of an evaluation.

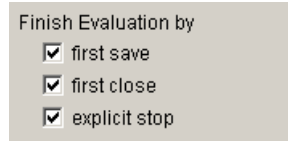


Figure 2-77 Finish Evaluation Pane

If you click **First Save**, the settings of the **Changes Afterwards** dialog box become active after saving the evaluation the first time. **First Close** activates the **Changes Afterwards** settings when you close the **Image** window during the initial evaluation. **Explicit Stop** allows you to mark the end of the evaluation anytime you want, using the **Stop Evaluation** button on the **Data Trailing** pane.

Automatic Export Pane

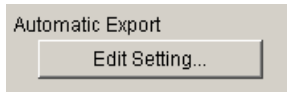


Figure 2-78 Automatic Export Pane

The **Edit Settings** button of the **Automatic Export** pane allows you to select, edit and save settings for automatically exporting the Profiles/Quantity Calibration/Runlength Calibration tables when the evaluation is saved to file.

Note – If you are perfoming evaluations with GLP support disabled, you can access the automatic export settings via the **Automatic Export** command of the **Options** menu.

Data Trailing Pane

After starting an evaluation, the controls of the **Data Trailing** pane are enabled.

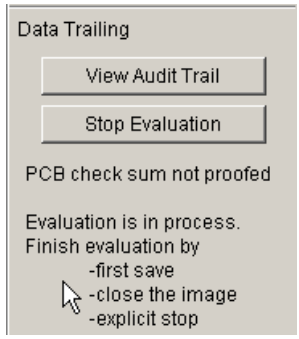


Figure 2-79 Data Trailing Pane (Evaluation in Progress)

The **View Audit Trail** button is used to open the **Audit Trail** window.

Use the **Stop Evaluation** button to mark the end of an evaluation anytime you want.

In the lower part of the **Data Trailing** pane, the status of the evaluation as well as the settings for ending the evaluation (depending on the checked **Finish Evaluation by** options) are given. In addition, if the PCB check sum option is disabled, a warning is displayed below the **Stop Evaluation** button.

After stopping the evaluation, the action which marked the evaluation's end is displayed (in this example by explicit stop via clicking the **Stop Evaluation** button)

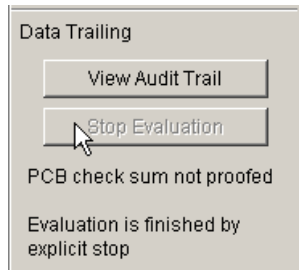


Figure 2-80 Data Trailing Pane (Evaluation Aborted by User)

Publishing Layout Toolbox in 1D TLC

In AIDA 1D TLC the standard AIDA **Publishing Layout** toolbox is expanded by four additional buttons for specifying the print options for the **Profiles**, **Quantity Calibration** and **Audit Trail**:

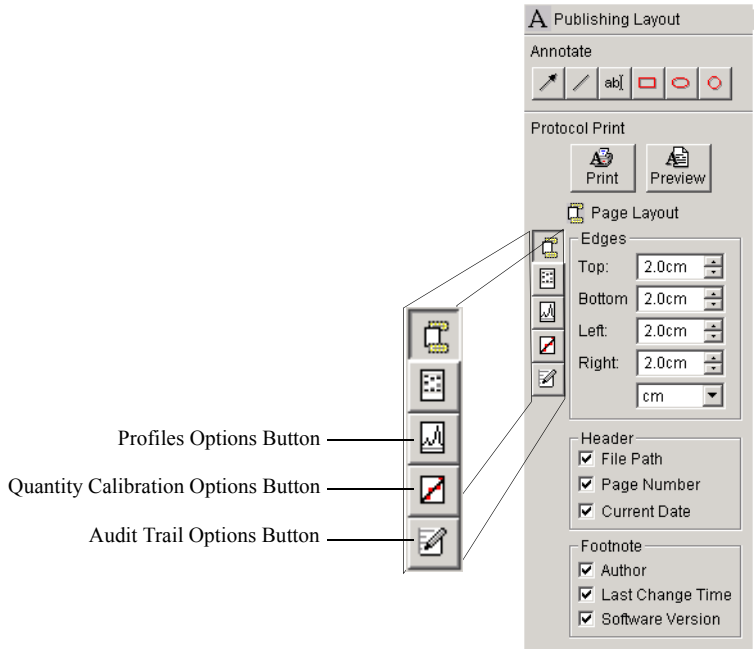


Figure 2-81 Publishing Layout Toolbox (Overview and Toolbar)

Print Options for Profiles

Using the **Profiles Options** button you can configure layout setting for the **Profiles** window.

1 Click the **Profiles Options** button.

The appearance of the **Protocol Print** pane changes.

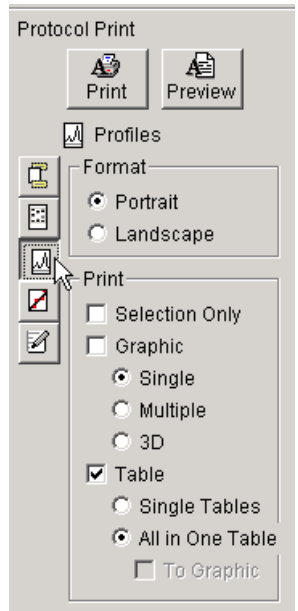


Figure 2-82 Print Options for the Profiles Window

2 Select the appropriate options:

In the **Format** pane select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button.

In the **Print** pane the scope for the print output can be specified:
Click **Selection Only** to print only the densitometers that are currently selected. **Graphic** selects the graphical representation of multiple selected profiles: **Single** shows each densitometer curve in a separate graphic, **Multiple** shows all (selected) curves on top of each other in a single graphic, and **3D** shows all (selected) curves in a 3-dimensional fashion within the same graphic.

To turn on printing of the result tables for the quantification and/or evaluation of the migration data, click to select the **Table** box. **Single Tables** prints a separate table for each 1D-densitometer, **All in one** prints all results combined in a single table. **To Graphic** prints the tables together with its associated densitometer graphic on a single page.

Print Options for Quantity Calibration

The second additional button of the **Publishing Layout** toolbox is used to configure the layout setting for the **Quantity Calibration** window.

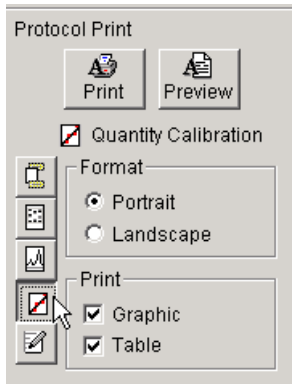


Figure 2-83 Print Option for the Quantity Calibration Window

As for the **Profiles** you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane. The options in the **Print** pane enable or disable the printing of the calibration curve (**Graphic**) and/or the calibration table (**Table**).

Print Options for Audit Trail

The last button of the **Publishing Layout** toolbox is used to configure the layout setting for the **Audit Trail** window in the **Protocol Print** pane.

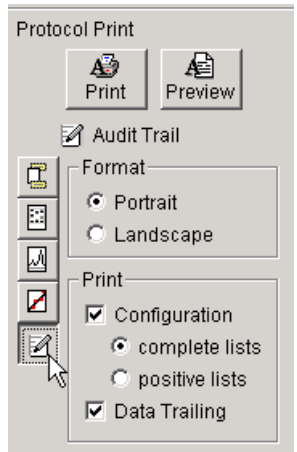


Figure 2-84 Protocol Print: Audit Trail Pane

As for the **Region Report** you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane.The options in the **Print** pane enable or disable the printing of the **Data Trail** settings (**Configuration**) and/or the recorded data from the **Audit Trail** window (**Data Trailing**). In addition, if the **Configuration** option is checked, you can choose to print the **Complete Lists** of settings or just the **Positive Lists** (the checked options only).

Print

In AIDA there are two ways for printing your image and evaluation data:

- Choose **Print** from the **File** menu,

  or

- Use the **Protocol Print** pane on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** pane provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Choosing the **Print** from the **File** menu opens the **Print** dialog box, allowing you to configure the printout to contain only the overlays, results and graphs that are currently needed. Click the checkboxes for the various items to turn them on or off. Select the number of copies, you want to print.

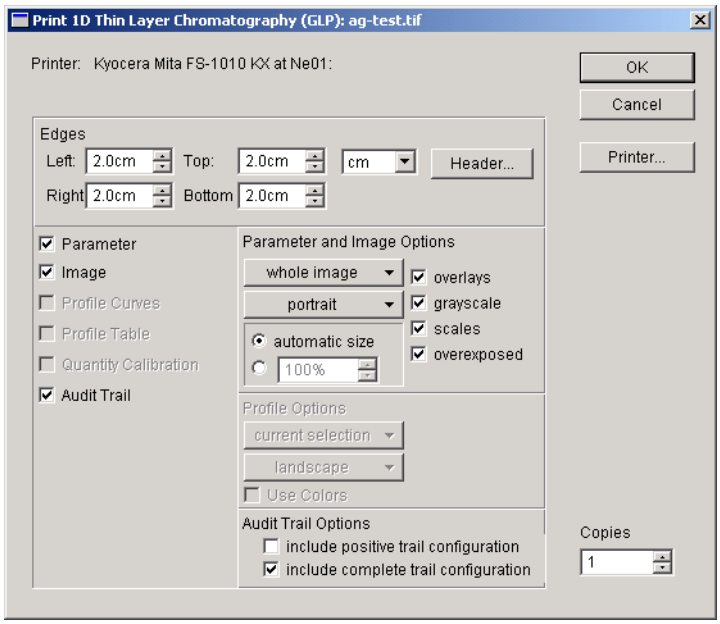


Figure 2-85 Print Dialog Box

The margins of the printout can be selected in the **Edges** part of the dialog box. The **Header** button displays the following dialog box:

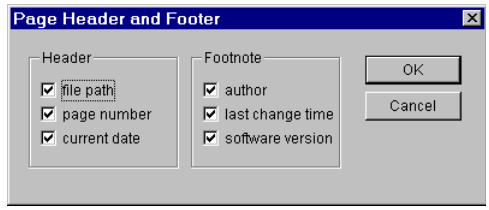


Figure 2-86 Page Header and Footer Dialog Box

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Image**, **Profile Curves**, **Profile Table**, and **Quantity Calibration** information can be selected in the left part of the **Print** dialog box.

In the right part of the dialog box (**Parameter and Image Options**) you can select **Whole Image**, or **Window Content** from the first drop-down menu. **Landscape** or **Portrait** format can be selected from the second drop-down menu. The printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be selected. The printout can be automatically fitted to the page, or the relative size of the image representation can be selected (**in %**).

In the **Profile Options** pane you can specify to print the **Current Selection** or **all profiles** as well as the paper orientation (**Landscape** or **Portrait**) for the printout.

Finally, using the **Audit Trail Options** pane, you can specify whether to include information about the **Data Trail** settings at all, and if yes, to which extent configuration information should be printed (**Include Positive Trail Configuration**/**Include Complete Trail Configuration**).

Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

- Using the **Print Preview** option of the **File** menu, or
- Using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** option of the **File** menu opens the **Preview** dialog box, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print** dialog box and those selectable from the various panes of the **Publishing Layout** toolbox. However, they are not stored with the data file.

The **Preview** option of the **Publishing Layout** toolbox allows you to preview all pages as configured in the **Publishing Layout** toolbox before they are actually printed. Clicking the **Preview** button opens the **Preview** window directly, since all layout setting are already made through the **Print Protocol** pane.

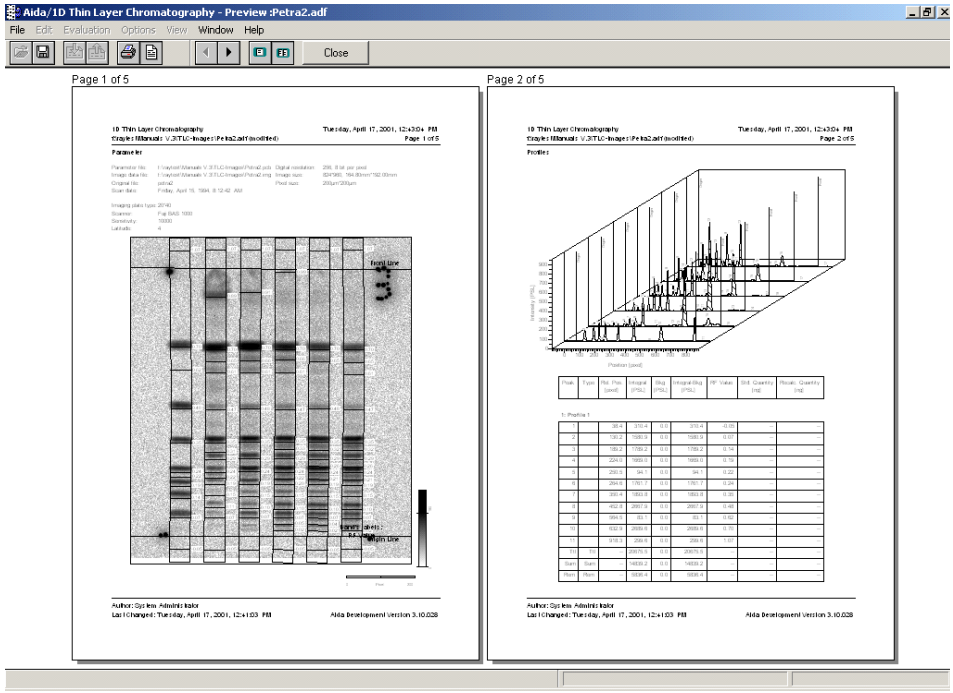

Aida/1D Thin Layer Chromatography - Preview :Petra2.adf
File Edit Evaluation Options View Window Help
Close
Page 1 of 5
Page 2 of 5

Figure 2-87 Preview Example

Export and Import

In AIDA 1D Evaluation various tables and graphs can be exported to be imported in other documents or programs. In addition the calibrations and templates can be exported to be applied to other images. The various functions can be found in the **Export** and **Import** submenus of the **File** menu.

Exporting Templates

To export your own evaluation templates:

1. On the **File** menu, click the **Export** menu item and then choose **Template** from the submenu.
2. In the **Export Template** dialog box that appears type a name for the export file and confirm the action by clicking **Save**,

or

1. Click the **Export Template** button on the toolbar to open the **Export Template** dialog box

Figure 2-88 Main Toolbar (Export Template Button)

2. Type a name for the export file in the **File Name** text box and complete the export action by clicking **Save**.

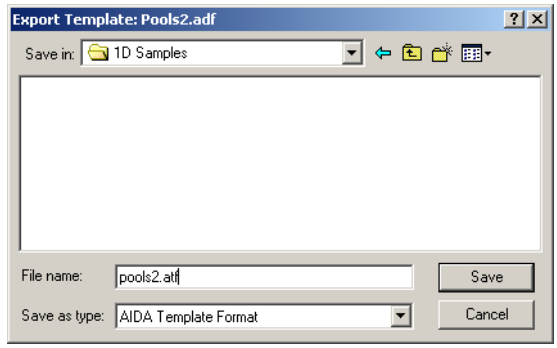


Figure 2-89 Export Template Dialog Box

Exporting Profiles

To export Profiles:

1 On the **File** menu, click the **Export** menu item and then choose **Profiles** from the submenu.

2 In the **Profiles Export** dialog box that appears type a name for the export file and specify the data format.

To export the graph in the Profiles window:

- Choose **Windows Metafile**.

  The graph is saved to a separate file in the Windows Metafile format.

To export the profile data of the Profiles window:

1 Select the **Profile Ascii File** data format.

2 Click **Save** to confirm the export.

  The **Profile Export** dialog box appears, allowing you to set further option for the export of the result table.

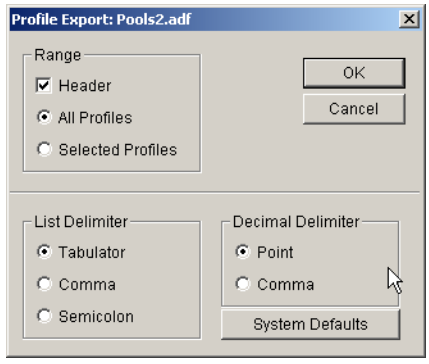


Figure 2-90 Profile Export Dialog Box

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

3 Complete the action by clicking **Ok**.

  The data are saved to a simple ASCII (text) file.

Exporting 1D Report Table

To export the 1D Report Table:

1. On the **File** menu, click the **Export** menu item and then choose **1D Report Table** from the submenu.
2. In the **1D Report Table Export** dialog box that appears type a name for the export file.

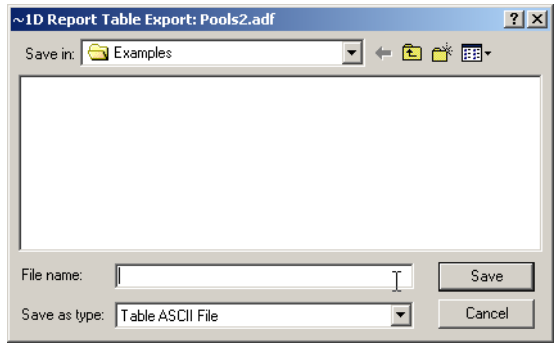


Figure 2-91 1D Report Table Export Dialog Box

3. Click **Save** to confirm the export.

   The **Profiles Export Options** dialog box appears, allowing you to set further option for the export of the result table.

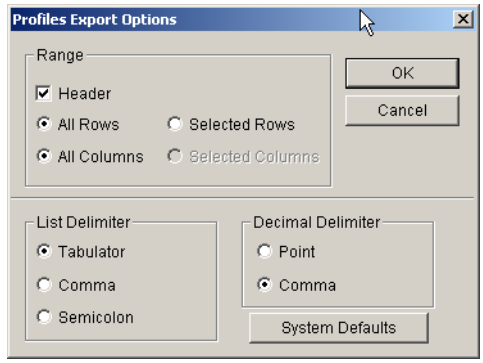


Figure 2-92 Profiles Export Options Dialog Box (1D Report Table Export)

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

4 Complete the action by clicking **OK**.

The data are saved to a simple ASCII (text) file.

Exporting Quantity Calibration

To export Quantity Calibration Data:

1 On the **File** menu, click the **Export** menu item and then choose **Quantity Calibration** from the submenu.

2 In the **Quantity Calibration Export** dialog box that appears type a name for the export file and specify the data format.

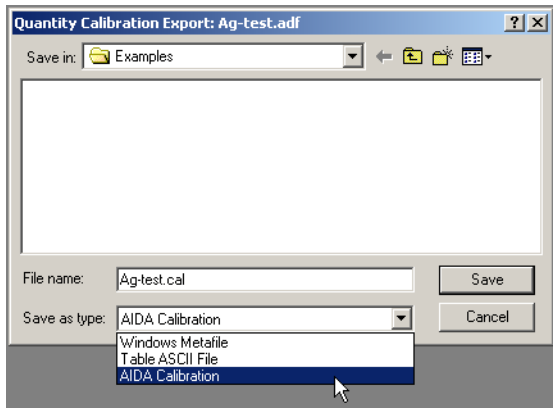


Figure 2-93 Quantity Calibration Export Dialog Box

To export the graph in the Quantity Calibration window:

- Choose **Windows Metafile**.

The graph is saved to a separate file in the Windows Metafile format.

To export the result table of the Quantity Calibration window:

1 Select the **Table Ascii File** data format.

2 Click **Save** to confirm the export.

The **Quantity Calibration Export Options** dialog box appears, allowing you to set further option for the export of the result table.

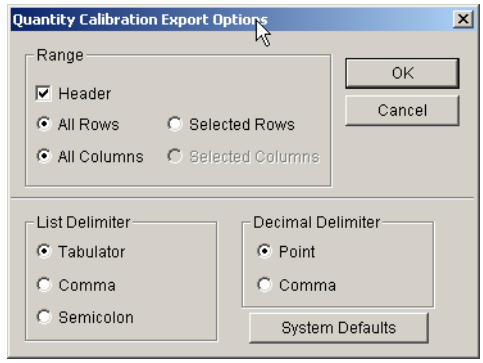


Figure 2-94 Quantity Calibration Export Options Dialog Box

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

3 Complete the action by clicking the **OK** button.

The data are saved to a simple ASCII (text) file.

In addition the calibration data can be exported to an AIDA Calibration file that can be imported into another image.

Exporting Audit Trail

To export **Audit Trail** data:

1 On the **File** menu, click the **Export** menu item and then choose **Audit Trail** from the submenu.

2 In the **Audit Trail Export** dialog box that appears enter a name for the export file.

3 Complete the export action by clicking the **Save** button.

The data from the **Audit Trail** window are exported to an ASCII file (**Audit Trail Ascii File**, `.txt`).

Importing Templates

To import previously saved templates to another equivalent image:

1. On the **File** menu, click the **Import** menu item and then choose **Templates** from the submenu.
2. In the **Import** dialog box that appears select the template to be imported and click **Open**.

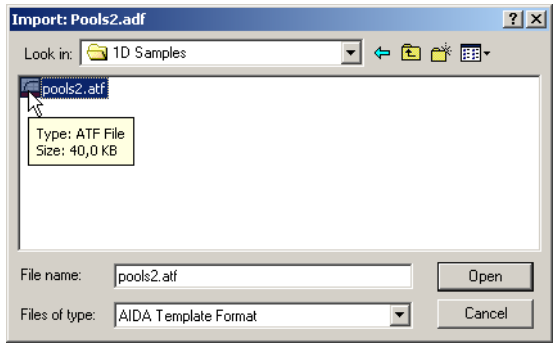


Figure 2-95 Import Template Dialog Box

The imported profiles can be arranged to fit the image by moving the whole array using the AIDA standard overlay editing functions.

Importing Quantity Calibration

1 To import the calibration curve from another image (e.g. taken at the same time with the same BAS screen, or from a second gel handled at the same time and captured under identical conditions), use the **Import** command of the **File** menu.

The **Quantity Calibration Import** dialog box appears allowing you to specify the calibration file (.cal).

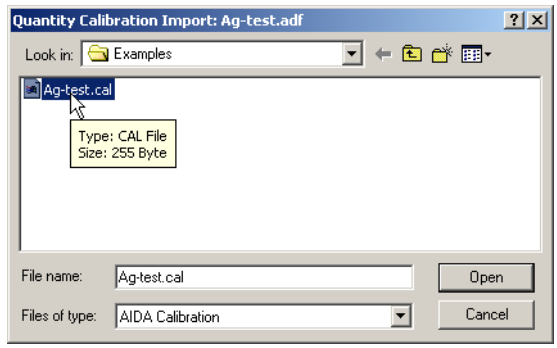


Figure 2-96 Quantity Calibration Import Dialog Box

Performing
1D TLC Evaluations 3

Setting the TLC Plate and the Origin/Front Line

The first thing to do when doing a 1D TLC evaluation is to set the TLC plate with the origin and front lines.

Consider the following image.

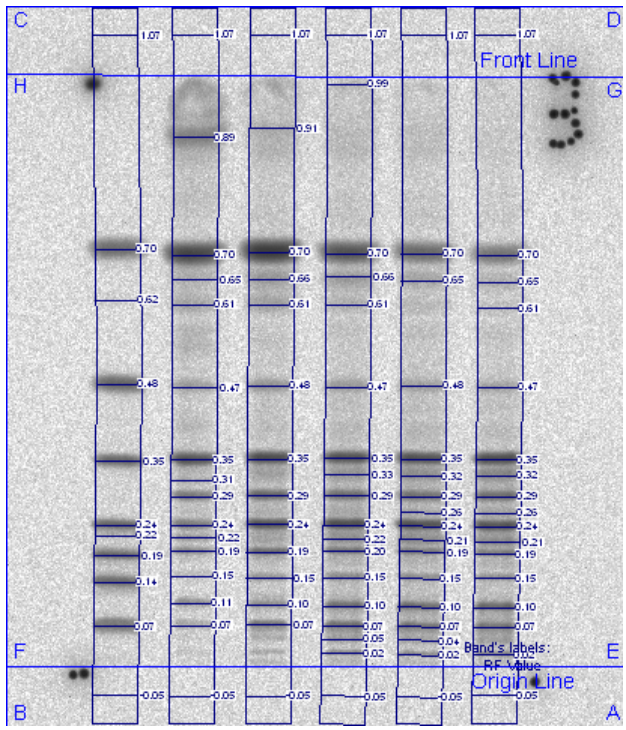


Figure 3-1 TLC Image Example

Here you can see the TLC plate overlay together with the origin and front line.

The TLC plate overlay is used for the calculation of the **Total** value shown in the **Profiles** window.

If the **1D TLC Calibration** toolbox is active, letters are displayed, referred to by dimension and position combo boxes of the toolbox.

You may change the location and size of the letters by choosing **Image Overlays** from the **Options** menu. Here you may also choose if the TLC overlays shall be drawn at all.

To set the TLC plate and the origin/front line, follow the steps below:

1. Select the **1D TLC Calibration** toolbox.
2. Select the run direction by choosing the appropriate entry from the **Run Direction** pull-down menu.

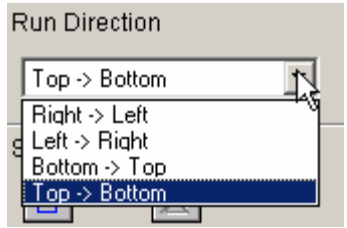


Figure 3-2 Run Direction Pull-Down Menu

3. Click the **Define Sample** button.

   This will create an overlay for the TLC plate, containing a second overlay for the origin and front lines.

Figure 3-3 Define Sample Button

   By default, the position of the TLC plate overlay's edges is congruent with the edges of the image.

If your TLC plate is not identical to the whole image:

1. Double-click near an edge of the image.

   This will activate the Editing mode for the TLC plate overlay.

2 Move, rotate and stretch this overlay until it is congruent with the TLC plate.

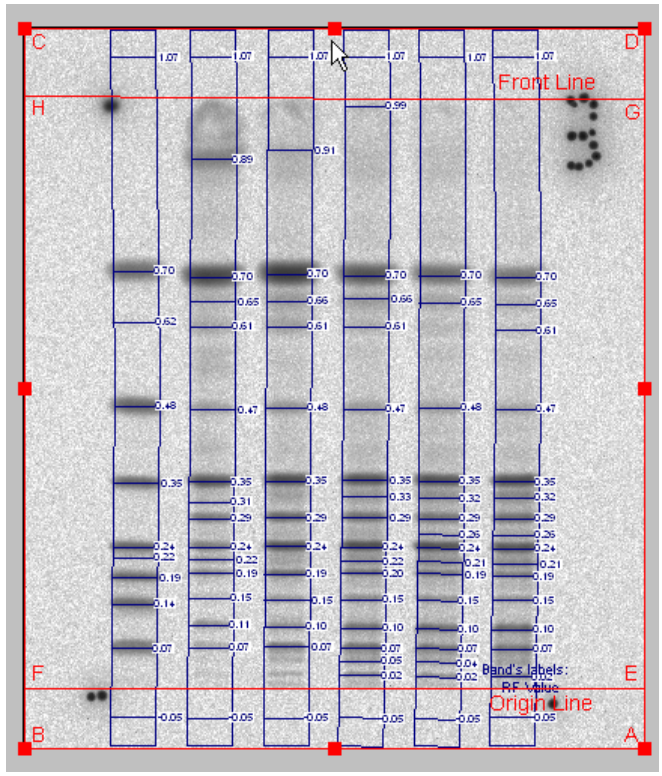


Figure 3-4 Editing TLC Overlay

3 Press the Return key to confirm the changes.

Adjusting Origin Lines

1 Double-click the origin line overlay.

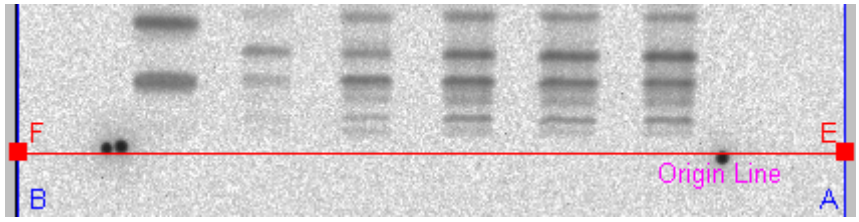


Figure 3-5 Editing Origin Line 1

2 Move, rotate and stretch it until it is congruent with the origin lines.

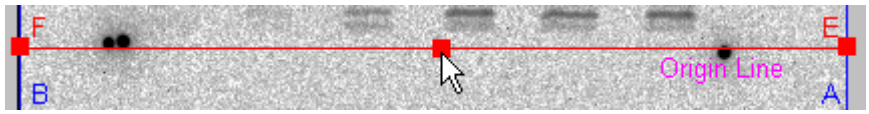


Figure 3-6 Editing Origin Line 2

In most cases, a horizontal line will be sufficient.

If the origin lines do not run parallel:

1 Double-click the origin line overlay again to enter additional vertexes.

2 Choose the appropriate **Line Model** from the respective pull-down menu to fit the overlay with the origin lines.

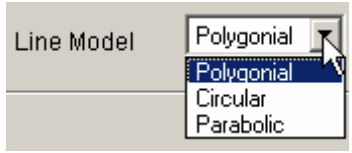


Figure 3-7 Line Model Pull-Down Menu

Note – The **Circular** and **Parabolic** line models are not available for line overlays with more than three vertexes.

---

3 Repeat the action for adjusting the front line overlay.

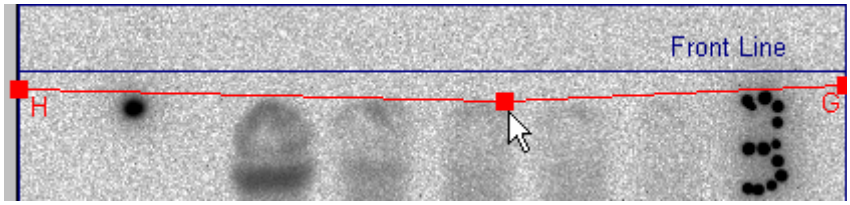


Figure 3-8 Adjusting non-parallel Origin Line

You can use the various combo boxes to type in directly the position and dimensions of your plate and origin/front line if you know them.

If you want to change the run directions after the overlays have been set, you have to delete the already set overlays first by clicking the **Delete Overlay** button and then redefine them.

Now you have completed the setting of the TLC plate and the origin/front line overlays.

You may proceed now with the usual 1D evaluation: Select the **Lane & Peak Determination** toolbox to assign lanes and bands and then use the **Quantity Calibration** toolbox to set quantity standards for normalization.

Assigning Lanes and Bands

The first step in the 1D evaluation is the detection/marking of the lanes, which is followed by the detection/marking of the bands, normalized by the setting of the quantity standards, as applicable. The result is a fully evaluated image where all the information can be collected in a comprehensive printout.

With AIDA 1D TLC it is usually preferable to select all parameters that are necessary for an automatic detection of lanes, background and peaks first with one example lane. When all parameters are well chosen, further lanes can be automatically selected and assigned. The detected bands can than be normalized by entering the values for the standard lanes (quantification standards).

To begin with the evaluation of lanes it is important to set the **Image Attributes** in the **Options** menu first (see AIDA Image Analyzer Basic Concepts and Features User's Guide). If these settings are not correct further image evaluation will not result in meaningful results.

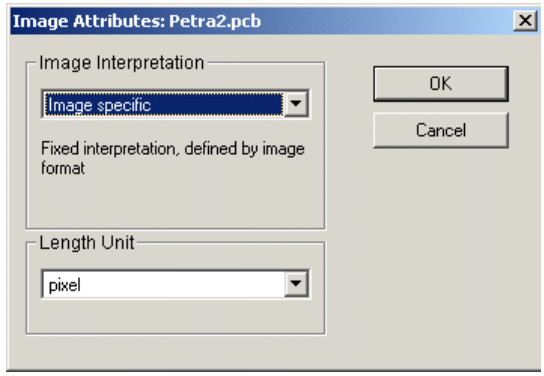


Figure 3-9 Image Attributes Dialog Box

When you use the **Lane & Peak Determination** toolbox for an application the first time, various parameters should be set.

1 First set the direction of the new lanes using the **Direction** pull-down menu.

2 Then determine the width of the lanes:

a. Select the automatic lane width measurement tool .

b. Click next to the upper left corner of a clearly visible band and drag to a position next to the lower right corner of the band. Release the button.

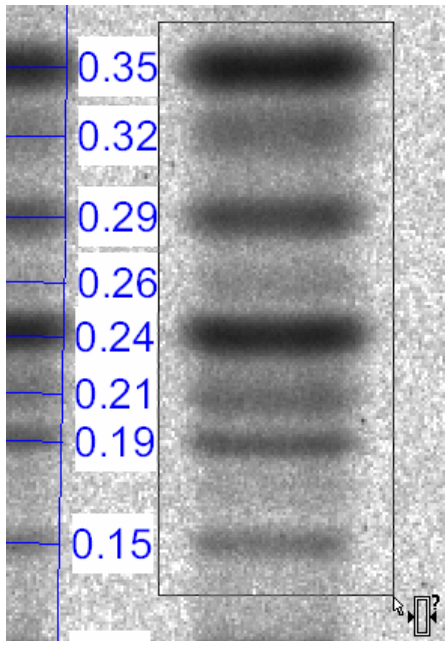


Figure 3-10 Selecting Lane

AIDA determines the width of the band and enters it into the appropriate combo box in the toolbox.

If no TLC plate overlay is set, the automatic lane width measurement tool uses the direction selected via the **Direction** pull-down menu. If a TLC overlay is set, the tool assumes the lane's direction to be parallel to the TLC overlay.

If the automatic lane width measurement does not lead to satisfactory results, determine the lane width using the manual width measuring tool:

1 Click this button , then click the left side of a lane and drag to the right side of the lane.

The width value is indicated.

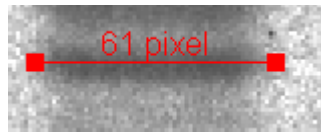


Figure 3-11 Lane Width Value Indicated by the Lane Width Measurement Tool

2 Release the mouse button and press the Return key or the right mouse button.

If you know the width of your lanes (e.g. from previous evaluations) you should type it directly into the combo box next to the lane width measurement tool buttons.

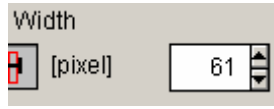


Figure 3-12 Width Combo Box

3 Activate one of the automatic lane determination tools:

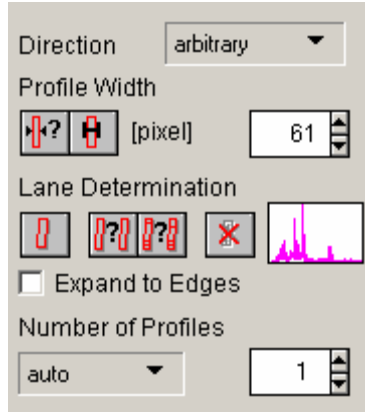


Figure 3-13 Lane Determination Tools

- To search for lanes only, select this button .
- To search for lanes and peaks simultaneously, select this button .

4 Click to the upper left corner of your lane, hold the mouse button and drag a rectangle to the lower right of the lane.

5 Release the mouse button.

AIDA will search for the lane (and peaks).

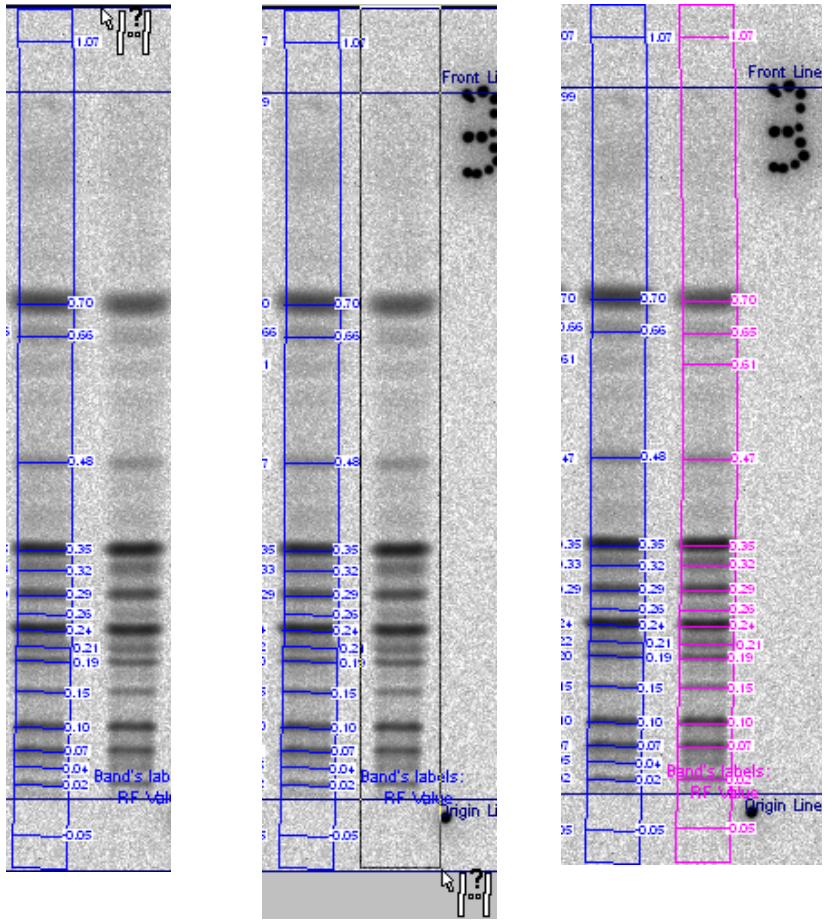


Figure 3-14 Searching for Lanes (And Peaks)

Images where the automatic lane detection does not work can be assigned manually:

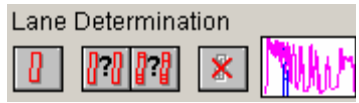


Figure 3-15 Lane Determination Tools

1 Activate the manual lane setting tool .

2 Click in the middle of the lane at the start point and drag the densitometer to the end of the lane.

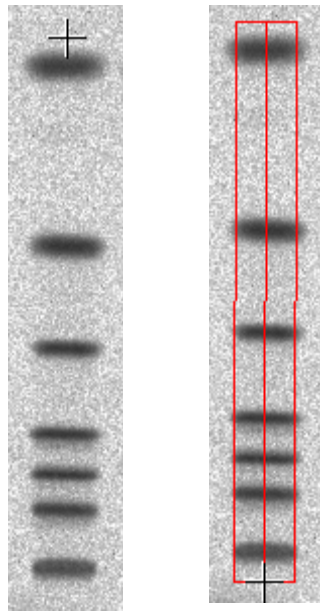


Figure 3-16 Determining Lanes Manually

Using this free densitometer function, every double-click on a position in the frame will create a new flexible point, which can be moved independently. This enables the creation of curved densitometer windows.

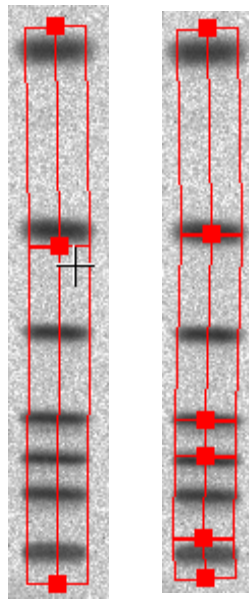


Figure 3-17 Creating Curved Densitometer Windows

Finally you can assign bands within profiles that were not automatically detected:

1. Activate the **Assign** function .
2. Click directly on the middle of the band.

If the automatic lane and peak detection tool does not lead to satisfactory results, determine the peaks using the controls of the Peak Determination panel:

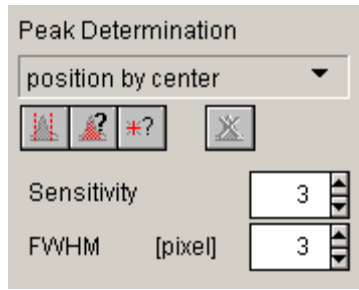


Figure 3-18 Peak Determination Panel

1 Set the appropriate option for marking the position of the band using the **Peak Determination** pull-down menu.

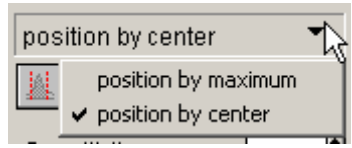


Figure 3-19 Peak Determination Pull-Down Menu

2 Activate the manual peak assignment tool .

3 Click on the start of the peak in the image or in the profile window, hold the mouse button and drag the second mark to the end of the peak.

You can change the peak region by dragging on one of the marks.

4 When the peak region is properly defined click the right mouse button or press the Enter key to confirm.

To delete currently selected peaks:

• Click this button .

To search peaks automatically within the active profile(s):

1 Set the **Sensitivity** and **FWHM** parameters to the appropriate values.

All these parameters should be tested out for a particular image type and can then be used for all further images of this type. The selected parameters can be saved under specific names using the **Preferences** option of the **Options** menu.

For FWHM you should choose the approximate full width at half max of the peaks to find.

2 Select this button to initiate the automatic search procedure.

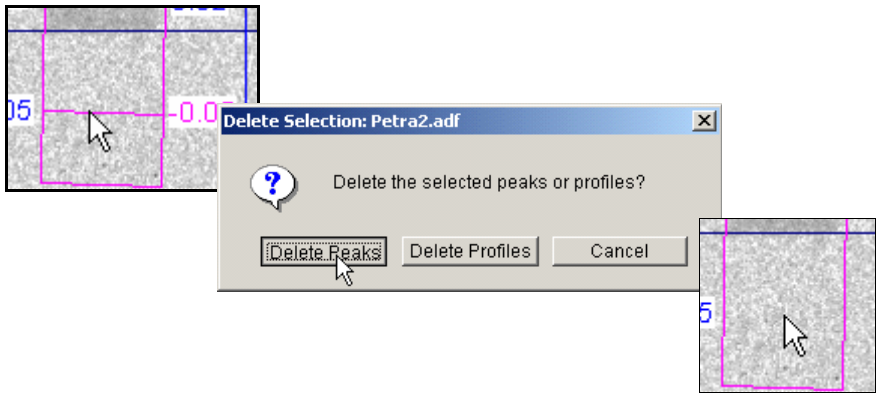
To search for individual bands:

1 Choose this button .

2 Click on the band.

Bands that are not found with any set of parameters can be double-clicked when this function is activated to obtain the retention factor for them.

Delete peaks of no interest by opening a rectangle over them in the selection mode and deleting the selected peaks by hitting the Del key on your keyboard or alternatively choosing **Delete Peaks** from the shortcut menu.



Check the **Profiles** window for peaks of interest.

Assigning Quantitative Calibration Standards

The profiles determined by the **Lane & Band Evaluation** function can be visualized by activating the **Profiles** command of the **View** menu. Any profile can be selected using the **Select Profiles** pop-up menu, or using the arrows under the **Profiles** window.

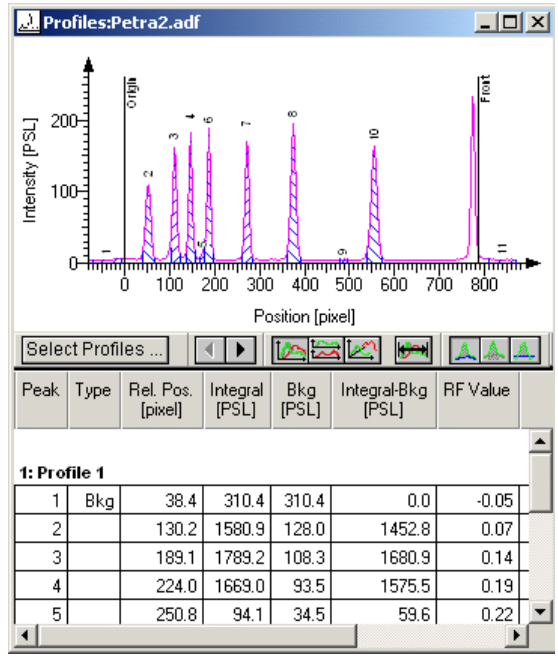


Figure 3-20 Selecting Profiles

After all peaks are properly determined, the quantity standards can be assigned:

1. Set the method for the intensity evaluation in the **Lane & Peak Determination** toolbox to **Intensity Summation**. **Optical Density** should be selected for color stained bands, **Intensity Summation** for radioactive, fluorescent stains and chemiluminescence applications, and **Averaged Intensity** can be used for fluorescent stains and chemiluminescence applications.

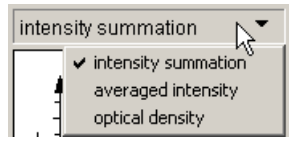


Figure 3-21 Setting the Intensity Evaluation Method

It should be noted that integration over the entire densitometer window should be done in sufficiently large densitometer windows to accommodate the entire band. Averaging can be performed best with densitometer windows that cover only the majority of the bands and omit empty space around them.

2 Assign bands representing a known quantity as standards for calibration by selecting the **Manual Assigment Tool** from the **Standard** controls of the **Quantity Calibration** toolbox and click on the appropriate band in the image, or the peak in the profile.

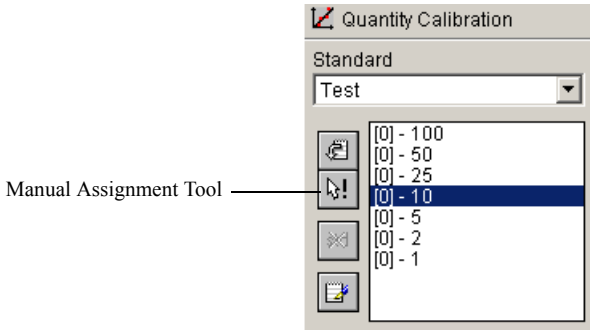


Figure 3-22 Manual Assigment Tool (Quantity Calibration Toolbox)

3 Enter the value and the dimension in the **Quantity Calibration** dialog box that appears.

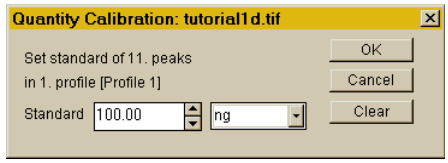


Figure 3-23 Quantity Calibration Dialog Box

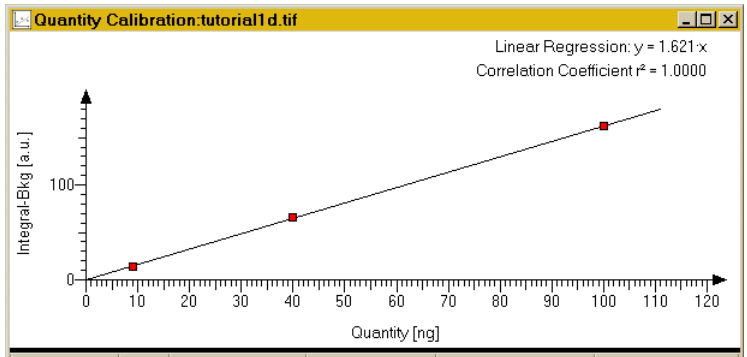
4 After all standards are entered, select the appropriate **Calibration Curve** type that fits the data (and application).

5 For most applications with properly assigned background, it might be advisable to select the **(0,0) Origin** option so that the standard curve goes through the origin.

6 Check the fit of the standard curve.

The r^2 value gives a clue about the fit; it should be very close to 1 for a good standard curve. The calibration curve can be selected on the basis of theoretical considerations or simply to fit the observed situation.

To evaluate the accuracy of the fit of the curve, you can check the difference between the quantity standard value and the recalculated standard value.



Profile	Peak	Integral-Bkg [a.u.]	Quantity Std. [ng]	Quantity Recalc. [ng]	Relative Error [%]
1: Profile 1	5	162.0	100.0	99.88	0.1158
11: Profile 11	1	65.4	40.0	40.34	0.8436
11: Profile 11	2	14.2	9.0	8.79	2.3641

Figure 3-24 Quantity Calibration Curve Example

Configuring the Evaluation Output

Following the calibration process outlined above, the entire image is calibrated and the results will be shown in the table pane of the **Profiles** window. One or more profiles can be displayed at the same time as outlined in the “Profiles Window” part of this manual.

It is important to use the **Retention Factor Normalization** function to get peaks of identical size at comparable positions.

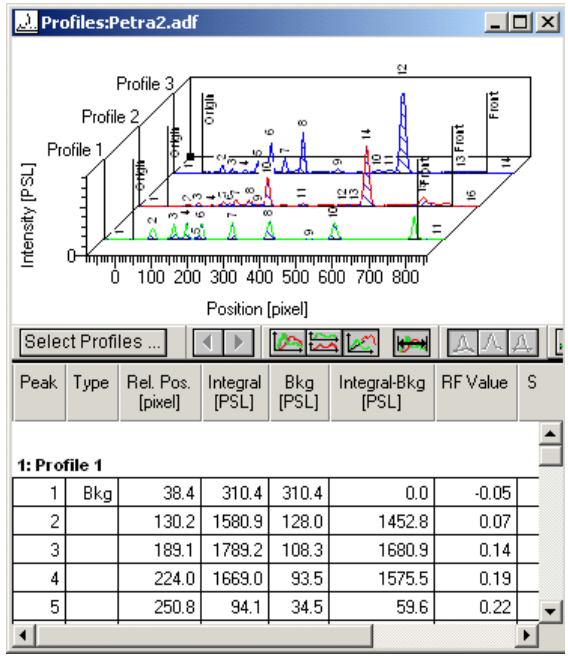

Profiles:Petra2.adf
Profile 3
Profile 2
Profile 1
Intensity [PSL]
0
0 100 200 300 400 500 600 700 800
Position [pixel]
Select Profiles ...
Peak | Type | Rel. Pos. [pixel] | Integral [PSL] | Bkg [PSL] | Integral-Bkg [PSL] | RF Value | S
1: Profile 1
1 | Bkg | 38.4 | 310.4 | 310.4 | 0.0 | -0.05
2 | | 130.2 | 1580.9 | 128.0 | 1452.8 | 0.07
3 | | 189.1 | 1789.2 | 108.3 | 1680.9 | 0.14
4 | | 224.0 | 1669.0 | 93.5 | 1575.5 | 0.19
5 | | 250.8 | 94.1 | 34.5 | 59.6 | 0.22

Figure 3-25 Retention FactorNormalization Example